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GUIDE TO THE STUDY
OF
ANIMAL PARASITES

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PREFACE

The subject of animal parasitology has in the past received but scant attention in the courses in zoology in this country. The student has had presented the general outline of the life history of the pork tapeworm of man, of the liver fluke of the sheep, and of trichina. As a rule he has seen only demonstration specimens, covering but a portion of the life cycles of these forms.

The present generation has seen a revolutionary change of attitude towards the subject. The remarkable discoveries of the relation of insects and their allies to animal parasites of man, the recognition that hookworm disease was widely prevalent in this country, and the extensive findings of other parasites of man and of animals which resulted largely from the hookworm campaigns, aroused a new interest in the parasites of man and of animals.

With this stimulus there has been an insistent demand for more attention to parasitic forms by departments of zoology. In many of our colleges and universities special courses in this subject are being offered and are being sought not only by premedical and veterinary students, but by those interested in public health work and animal husbandry. Increasing numbers of students of general zoology are finding in such courses an introduction to some of the most fascinating problems in their chosen field of work.

The demand for this special work has brought its problems to the teacher. In many cases he has been at a loss as to where and how to obtain material for his rapidly growing classes. Unlike the standard materials of the first-year course, it was not to be found in the regular biological supply houses. There was a general feeling that it was rare and inaccessible to the average department, and there was little idea as to what suitable forms were available from domesticated or wild animals. There has been almost complete dearth of suitable texts and at the present time there is little choice in the way of a laboratory guide for a general course in animal parasitology.

Under these circumstances the writers have felt that there is a place for the "Guide to the Study of Animal Parasites" which is here offered. It is the outgrowth of fifteen years of experience on the part of the senior author in presenting the subject to a group of students with varied interests, such as is to be found in most of our schools. It is hoped that the suggestions as to sources of material will considerably lighten the work of the non-specialist who is called upon to present the subject. They are also intended to aid the independent student of zoology in getting an introduction to the field of parasitology. It will be noted that the introductory study of the incidence and distribution of the animal parasites of the frog is followed by the study of the trematodes and that consideration of the protozoan parasites is reserved for the later periods of the course. We have found this the most feasible sequence for classes with a background of only general zoology, but some instructors may prefer to reverse this treatment.

The omission of the arthropods from such a text needs explanation. In most of the schools of this country where the subject of animal parasitology is taught, the work in medical and veterinary entomology is presented in a separate course. In any event, there are available excellent outlines for the study of the elements of entomology. The protozoologic and helminthologic aspects of the relation of arthropods to disease are those which cannot be presented satisfactorily without more of a background than most of the courses in medical entomology provide.

It is with much reluctance that the authors have restricted the references to literature to so small a number and these almost exclusively in English. The original plan of citing original monographs regardless of place of publication was discarded because it was evident that most of these would be inaccessible outside of research centers. The papers cited contain, in most cases, special bibliographies, and many of the comprehensive texts listed in the Appendix include very extensive references to the literature. The student should be urged to consult original sources and should have emphasized the importance of preparation in modern languages if he expects to do advanced work in any field of zoology.

A section on the essentials of technique of collecting and preparing animal parasites is appended. In addition there is given a list of the more important parasites of laboratory animals which will not only be of aid to the instructor in locating material but will serve as a starting point for special survey work by the interested student.

The senior author particularly wishes to acknowledge his indebtedness for suggestions by W. L. Chandler, Laurene Krogh, Gerard Dickman, and Nordahl Peterson, former student assistants who were associated with him in presenting this course. The efficient services of Miss Grace E. Jones in preparing, redrawing, and arranging illustrations also deserve special mention. To the various publishers and authors who have granted ready permission to use illustrations, thanks are due.

THE AUTHORS.

MINNEAPOLIS, MINN.
January, 1930

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INTRODUCTION

The laboratory exercises outlined in the following pages are designed to introduce the student to the study of animal parasitology. Normally, the work will be undertaken after a preliminary course in general zoology or biology and it is thus unnecessary to discuss at length the equipment to be used or methods of study.

A knowledge of the use of the compound microscope is assumed, though any user of the instrument would profit by reading over carefully the booklets on this subject which are furnished by the makers. Certain elementary rules are so commonly ignored, even by advanced and graduate students, as to need further emphasis.

Care of the Microscope.—It must be remembered that the microscope is an instrument of precision and should be treated as such. It should be protected from dust and from fluids. The glass surfaces of the lenses must not be touched with the fingers for that will soil them. There is probably no elementary rule of microscope manipulation which is more commonly violated, yet its validity is obvious.

Students often have difficulty in determining the location of dirt and films which cause indistinctness of the image. If the fault is in the preparation the fact will be obvious if the slide is observed carefully while being moved; if in the eyepiece, by rotating it while looking through the microscope; if in the mirror by moving it.

Lenses should be cleaned carefully by breathing on them and wiping with special lens paper (Japanese filter paper) or a clean soft linen rag. If this is not sufficient moisten the edge of the rag slightly with alcohol. If the cloudiness should be due to balsam or an oily substance, it will be necessary to use a small amount of chloroform or xylol but this must be done quickly to obviate the possibility of these fluids penetrating and injuring the setting of the lens.

Care of Preparations.—In cleaning the cover glasses of prepared slides be very careful not to exert pressure as the chances are that the slide will be ruined, even though it does not show injury. Microscope clips must not be placed over the cover glass for they, too, will cause movement of the mounting medium and irreparable injury to the preparation.

Do not attempt to use high power lenses on thick preparations such as those of trematodes, tapeworm proglottides and the like—the proper manipulation of the mirror and the iris diaphragm are important factors in bringing out detail under the low power lenses.

Use of Oil Immersion Lenses.—In the study of parasitic protozoa it is often necessary to use the oil immersion objective. This is, as its name implies, an objective whose front lens is immersed in a special cedar oil of the same refractive index. As it has a very short working distance it can be used only on preparations having very thin cover glasses or on dried uncovered films with only the oil. Special precautions must be observed to prevent injury of either the lens or the preparation. A small drop of oil is applied directly to the lens or placed on the preparation and the lens lowered until it is in contact with the fluid, the observer watching closely meanwhile with the head at the level of the stage. Focus cautiously—if there is apparent movement of tissues in a balsam mount it is a danger signal that the cover glass or the layer of balsam is too thick and the lens must immediately be focussed upwards.

Dissecting Microscopes.—The modern binocular dissecting microscopes are a great aid to the student of parasitology. An equipment of lenses giving a range of magnification of from seven to thirty times, is the most satisfactory. The higher magnifications are of little use in general work.

The student, however, must not get the idea that these are essential for his work. Most of the anatomical details discussed in the following pages were worked out long before the binocular dissecting microscopes were invented. Some simple type of dissecting stand, even if only the tripod with a lens which is focussed by screwing up and down in the metal frame can be provided in any laboratory. Skill in dissection of minute objects under the ordinary compound microscope is readily acquired and even today is an essential equipment of the student of zoology.

Moreover, every student of the subject should own and habitually use a good magnifier, mounted in a folding pocket case. While the higher-priced lenses are of course preferable, very good service is given by the cheaper doublet type. The magnifying power should not exceed 10 to 14 diameters.

Dissecting Instruments.—The ordinary dissecting set as required in the elementary course in zoölogy is sufficient for this course. Heavy scissors with one blunt probe point, or the regular enterotomy scissors are very useful in the examination of animals for intestinal parasites. Bone-cutting forceps should be available. For examination of large animals special equipment of skinning knives and other tools is necessary.

Preservation and Mounting of Specimens.—It will add much to the value of the work if the student will take advantage of opportunities to examine various animals for parasites. These can be preserved by the methods given in the appendix. In every case care should be taken to preserve accurate data as to the host and the organs from which the specimens were taken and the date and locality.

GUIDE TO THE STUDY OF ANIMAL PARASITES

CHAPTER I

THE MAJOR GROUPS AND DISTRIBUTION OF ANIMAL PARASITES AS ILLUSTRATED BY THE ANIMAL PARASITES OF THE FROG

TECHNICAL SUGGESTIONS

At the outset of the work, when the general principles of animal parasitism are being discussed in the lectures, two laboratory periods may be devoted profitably to a general survey of the parasites of the frog. For these practicums freshly killed frogs of any species may be used. It is not necessary that they be large enough for ordinary anatomical work. When feasible it is advantageous to have them from different habitats and, preferably, recently caught. Shortly before the class period they should be chloroformed.

For purpose of future study and statistics it is well at the close of the period to collect the specimens found and to preserve them, with accurate data as to sources, according to the directions given in the Appendix. If time and facilities permit, it is still better to have this done by the students themselves.

SYSTEMATIC REVIEW

Before undertaking the practical work outlined, the student should review the characteristics of the animal phyla and classes with which our course will be chiefly concerned.

Phylum PROTOZOA.—Animals in which the entire body consists of a single cell which, however, may possess a highly complicated structure. Four classes are usually recognized.

Class RHIZOPODA.—Protozoa in which the motile organs are pseudopodia.

Class MASTIGOPHORA.—Forms possessing an outer cell integument and flagella as motile organs.

Class SPOROZOA.—Parasitic Protozoa typically without organs of locomotion; reproduce by spore formation. Group not a natural one.

Class INFUSORIA.—Protozoa with an outer cell integument; always ciliated either through life or in the young condition.

Phylum PLATYHELMINTHES.—The platodes, or flatworms. Bilaterally symmetrical animals devoid of true metameric segmentation and without body cavity. There is no blood-vascular system, but an excretory (water-vascular) system is present.

Class TREMATODA.—Flukes. Parasitic flatworms without a covering of cilia in the adult state; with a well-developed digestive apparatus typically with a single opening, the mouth, at the anterior end of the body. The sheep liver-fluke is an example.

Class CESTODA.—The tapeworms. Endoparasitic flatworms, without cilia and without a digestive cavity; usually becoming segmented as they mature.

Phylum NEMATHELMINTHES.—The roundworms, or threadworms. The body is cylindrical, spindle-shaped, or thread-like, unsegmented, and covered with a thick cuticle; the body cavity (not a true cœlom) is usually spacious. The sexes are usually separate but hermaphroditic species occur.

Class NEMATODA.—With an alimentary canal but without a proboscis. Both free-living and parasitic forms.

Order EUNEMATODA.—Alimentary canal typically complete and present throughout life. Familiar examples are the ascaris worms, pinworms, hookworms.

Order GORDIACEA.—The so-called "hair-snake." Larval stages parasitic and possessing an alimentary canal. Adults free living, without an alimentary canal.

Class ACANTHOCEPHALA.—The thorny-headed worms. Lacking alimentary canal; possessing a protrusable proboscis which is covered with many rows of recurved hooks.

Phylum ANNULATA.—The segmented, or annelid worms. Body composed of similar segments, without jointed legs; alimentary canal with two openings. Only one class contains a considerable number of parasitic forms.

Class HIRUDINEA.—The leeches. Segments marked externally by secondary rings. Each end of the body is furnished with a sucker.

Phylum ARTHROPODA.—Crustaceans, mites, ticks, and insects. Bilaterally symmetrical animals in which the body is segmented and bears a pair of jointed appendages on each or some of the segments. Not considered in this course.

PRACTICAL WORK

The object of this exercise is to illustrate the common types and general distribution of parasites in the organs and tissues of an animal host. For this purpose the frog is an exceptionally favorable subject. It harbors a large number and variety of entozoa, illustrative not only of the major groups but even of genera of important parasites of man. The work of this practicum is of a general nature, but we shall return to the study of frog parasites in greater detail as we take up particular groups.

Lay the frog on its back on a dissecting board or tray and extend and pin down the legs. Open the abdominal and thoracic cavities by a median ventral incision and pin back the flaps. By way of review identify the general internal anatomical structures before making the special study of them for parasites.

Examination of the Blood.—Using slides and covers which have been thoroughly cleaned in alcohol to remove all grease, mount a small drop of blood. Cut down the light in the microscope and carefully search through the entire field for blood parasites. Care should be taken not to make the mount too thick, thereby rendering it opaque. If necessary a drop of physiological salt solution (0.75 per cent NaCl) may be added to dilute the blood.

Trypanosoma rotatorium is a flagellated protozoan found free in the blood plasma. It is approximately twice the length and about the breadth of the red corpuscles of the frog, moves about actively, and presents marked changes in form. It is rarely abundant but, if present, can usually be found in the blood from the kidney.

Lankestrella ranarum is a sporozoan which, like the parasite of malaria, is found within the red blood corpuscles and blood plasma of the host. In the fresh corpuscle it appears as a clear, spindle-shaped body. Spindle- or crescent-shaped forms predominate in the blood. Another sporozoan, *Dactylosoma ranarum,* also occurs in the red blood cells of frogs and undergoes an asexual multiplication within them. If heavily infected frogs are found, permanent preparation may be made as directed in the Appendix.

Blood filaria, minute larval roundworms, have been reported for frogs but have not been recorded for our native species.

Body Cavity.—*Clinostomum attenuatum.*—Examine the mesenteries, the peritoneum of the body cavity, and the lymph spaces between the skin and muscles of various parts for little cream-colored nodules, about the size of a pinhead. If such are noted, carefully tease one open and there will be found a larval fluke, which is capable of developing to maturity in various aquatic birds that prey on frogs. Other undetermined larval flukes have been reported in the muscles of frogs; and the sporozoan, *Glugea danilewskyi*, has also been noted. It occurs as white striations in the muscles, containing spherical granules and many spores.

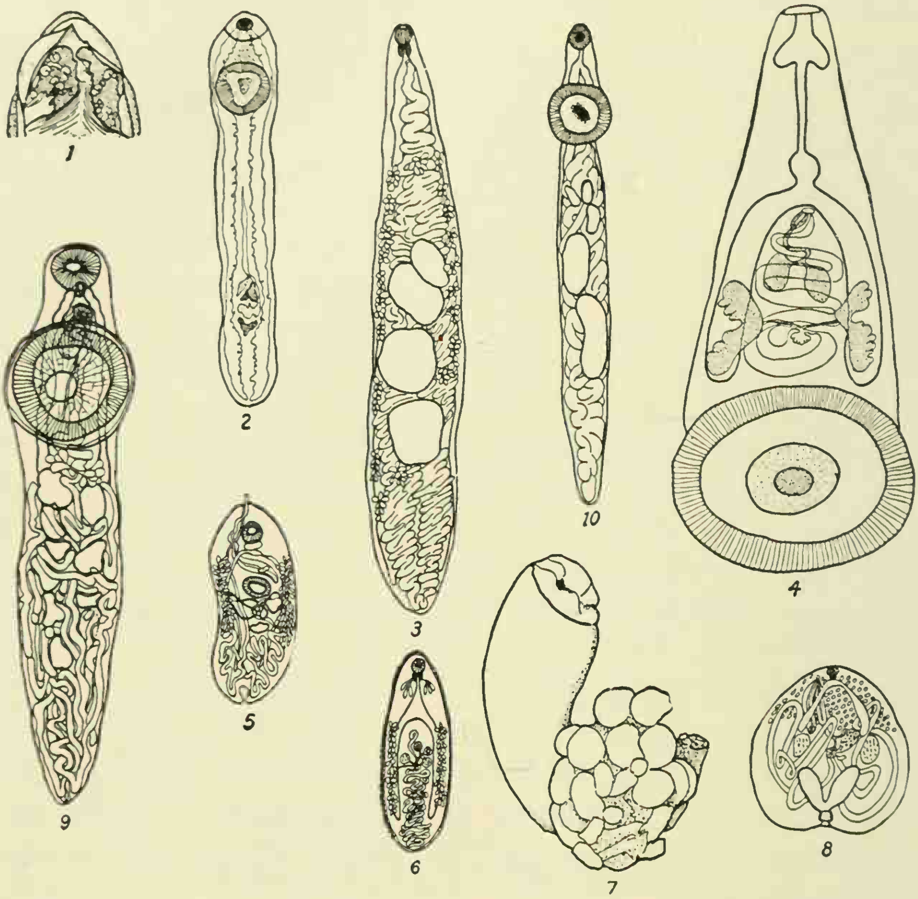

Fig. 1.—Recognition sketches of common trematode genera from native frogs. Magnification (except 1 and 7) 10 ×. 1, *Clinostomum* larvæ under skin of throat; 2, *Clinostomum*, isolated; 3, *Pneumonœces* from lung; 4, *Diplodiscus;* 5, *Glypthelmins;* 6, *Cephalogonimus;* 7, cysts of *Loxogenes* on pyloric end of stomach; 8, *Loxogenes;* 9, *Gorgodera;* 10, *Gorgoderina.*

Lungs.—Remove the lungs and tease them apart in a dish of physiological salt solution. Probably both roundworms (Nematoda) and flukes (Trematoda) will be found. Note the number of each.

Rhabdias ranæ is a nematode measuring from 3.5 to 5 mm. in length which is commonly found in the lungs of American frogs.

Rhabdias bufonis, an European species measuring from 11 to 13 mm. has been reported for our native frogs as well. Both species are viviparous and their larvæ may be found in the alimentary canal of the frog.

Pneumonœces sp.—At least six different species of flukes belonging to this genus are to be found in the lungs of North American frogs. Mount a specimen and note under the microscope the characteristic suckers. The dark brown eggs, present in enormous numbers, obscure most of the anatomical details. The discharged eggs, with a characteristic cap, will be noted later in the intestinal contents.

Surface of Abdominal Organs.—*Loxogenes arcanum* is a small trematode living in thick-walled closed cysts on the stomach, liver, and bladder of various frogs. Two individuals occur in each cyst.

Nematodes.—Several imperfectly known nematodes occur as immature larvæ in cysts on the walls of the stomach and various other organs.

Alimentary Canal.—Beginning at the mouth, slit the alimentary canal, examining carefully for various parasites.

Flukes.—*Halipegus occidualis* is a moderate-sized fluke with powerful suckers, which is reported from the mouth and Eustachian tube of a frog.

Glypthelmins quieta occurs in the intestine, as does *Cephalogonimus americanus.* These two species are small flukes superficially resembling each other very much. They are both cylindrical, cigar-shaped flukes measuring about one-eighth of an inch (3 mm.) in length. A positive differentiation of the species will not be attempted at this time.

Diplodiscus temperatus, found in the rectum, is recognized by the fact that the enormously developed ventral sucker is at the caudal end of the body.

Nematodes.—Several species of nematodes may be found in various parts of the alimentary canal. They should be examined, but further study will be postponed.

Cestodes.—Several species of tapeworms have been reported as adults in the intestines of frogs. Another lives as an encysted larva in the muscles.

Protozoa.—Numerous Protozoa, representing each of the four classes, are to be found in the alimentary canal and will be studied more in detail at a later practicum. The following should be especially sought at present, by mounting a little of the black content of the rectum in salt solution. An excess of water should be avoided. In order to get the best results the mount should be sufficiently transparent to read ordinary print through it.

Opalina is a ciliated protozoan so large as to be visible to the naked eye as a white dot. It is greatly flattened, covered by minute cilia which give the surface distinct oblique striations. Numerous species have been described for frogs and other batrachians.

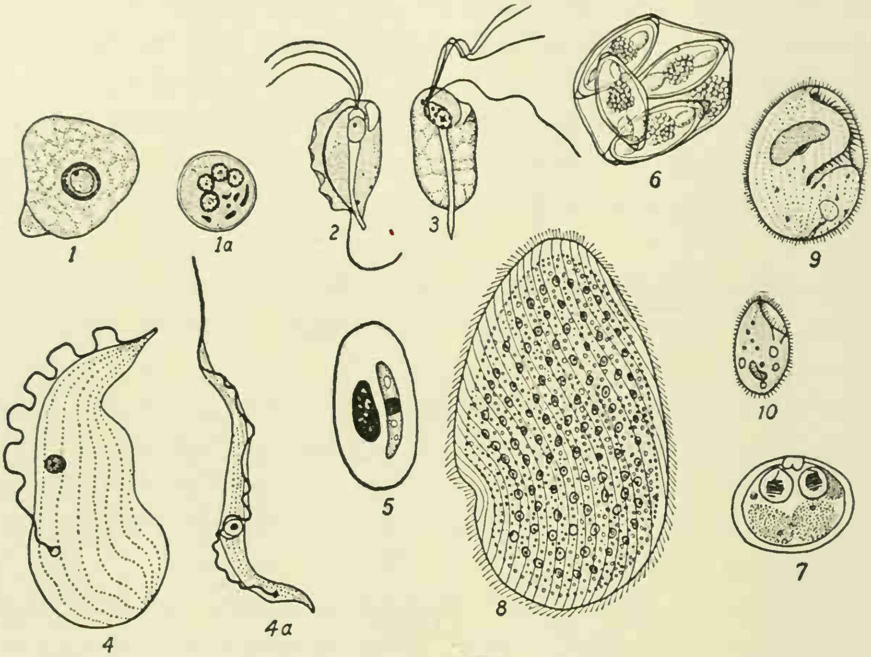

Fig. 2.—Recognition sketches of genera of common protozoal parasites of frogs; except as noted, all from the intestine. 1–7, drawn to the same scale; 8–10 to one-third. 1, *Endamœba;* 1*a*, *Endamœba* cyst; 2, *Trichomonas;* 3, *Trichomastix;* 4, 4*a*, *Trypanosoma* from blood; 5, *Lankestrella* in nucleated red cell; 6, *Eimeria* cyst containing four spores; 7, *Leptotheca* spore from kidney; 8, *Opalina;* 9, *Nyctotherus;* 10, *Balantidium.* (*Adapted from various authors.*)

Balantidium entozoön is of especial interest because of its close relationship to *Balantidium coli,* which is a serious parasite of man. It is larger than the free-living Paramecium studied in courses in general zoölogy and has a terminal gullet, lined by cilia much coarser than those of the rest of the body.

Nyctotherus cordiformis is similar to *Balantidium entozoon* but is readily distinguishable by the presence of a gullet running obliquely across the body rather than a terminal gullet as in the latter species.

Mastigophora ("Flagellata") may also be found in abundance. The larger tadpole-shaped individuals belong to the species *Trichomonas batrachorum,* a genus represented among the parasites of man.

Endamœba ranarum is a representative of the Rhizopoda which is not uncommon in frogs. It very closely resembles some of the amœbæ of man. Under favorable conditions motile and encysted forms may be found.

Eimeria ranæ is a sporozoan closely related to the coccidian which causes fatal epidemics among rabbits and other mammals. The oöcysts of the *E. ranæ* may often be found in the contents of the large intestine.

Bladder.—*Gorgoderinæ.*—Some half dozen species of flukes belonging to the genera *Gorgodera* and *Gorgoderina* are to be found in the bladder of frogs.

Polystomum integerrimum.—This interesting fluke, characterized by the possession of six suckers at the caudal end, is common in the bladder of frogs in Europe. Related species are occasionally found in our native frogs.

Kidney.—*Diplospora lieberkühni* and *Leptotheca ranæ* are sporozoans, which have been found in the kidneys of various frogs.

Directions for the preservation of parasitic worms and Protozoa and for the preparation of permanent slides will be found in the Appendix.

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CHAPTER II

THE MORPHOLOGY OF THE TREMATODA, OR FLUKES

TECHNICAL SUGGESTIONS

For demonstration of the monogenetic flukes, species of the well-known genus *Polystomum* may be found in the mouth and the urinary bladder of various turtles, or in the urinary bladder of our native tree frogs. Specimens of the best-known species, *P. integerrimum*, can be purchased from European dealers. The minute **Gyrodactylidæ** may be secured from the gills and skin of various fish.

For study of the anatomy of a digenetic fluke, the commonly used sheep liver fluke is so complicated as to be wholly unsuitable for beginners. Little better are the various lung flukes of frogs which are often substituted. Much more suitable species are *Opisthorchis pseudofelineus* from cats and *Clonorchis sinensis* from the liver of man. Prepared slides of the latter are now readily available from dealers and we shall use them as a type. In default of both, the immature *Clinostomum* from the flesh of perch and other fish, or from frogs, may be used. Study of the simpler forms may be followed by that of the frog lung flukes and the sheep liver fluke.

CHARACTERISTICS OF THE TREMATODA

The **Trematoda,** or flukes, are exclusively parasitic flatworms (*Platyhelminthes*), often leaflike in shape, without a covering of cilia in the adult state. They possess a well-developed alimentary canal with but one opening, the mouth, at the cephalic end of the body. Suckers are developed on the ventral surface and in the region of the mouth, their position and structure being of much systematic value. With rare exceptions (blood flukes) trematodes are hermaphroditic.

The class is divided into two subclasses, the **Monogenea** and the **Digenea.** As indicated by the names, the monogenetic flukes develop directly, on a single host, while the digenetic forms require two or more host species for their development.

PRACTICAL WORK

Polystomum sp.—As an illustration of the more primitive flukes, or Monogenea, there will be demonstrated a representative of the genus *Polystomum* from frogs or turtles. Note the six posterior suckers, the small hooks, and the two large hooks, the branched alimentary canal, and the short uterus containing a single egg. Make a sketch showing these points.

Clonorchis sinensis, the Asiatic liver fluke of man, or a related species will be taken as a representative of the digenetic flukes. Study under low power the prepared slide furnished and note the following features:

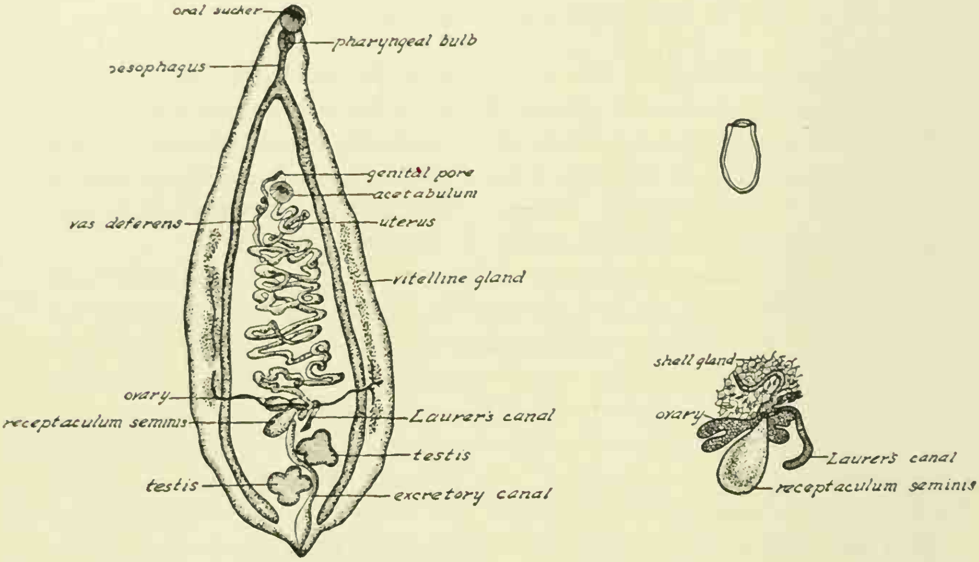
oral sucker
pharyngeal bulb
oesophagus
genital pore
acetabulum
vas deferens
uterus
vitelline gland
ovary
receptaculum seminis
Laurer's canal
testis
testis
excretory canal
shell gland
ovary
Laurer's canal
receptaculum seminis

Fig. 3.—Anatomy of a trematode, *Opisthorchis viverrina*. (*Barker, after Poirier.*)

External Characters.—Shape and size. Is the cuticle smooth or spinose? (In order to determine this, cut down the iris diaphragm of your microscope and examine the margins of your specimen.) What is the number, position, and relative size of the suckers? What is their relation to the mouth? Close to the median anterior margin of the ventral sucker is the genital pore, the common outlet of the male and female organs. Locate the excretory pore at the caudal tip of the body.

Alimentary Canal.—Distinguish the *mouth*, the muscular *pharynx*, the short, unbranched *œsophagus*, and the two branches, or *rami*, of the alimentary canal. How far caudad do these rami extend? Do they exhibit lateral branches, or diverticula? Is there an anus present?

Reproductive System.—As in the great majority of flukes, *Clonorchis* is hermaphroditic; that is, both male and female organs are found in the one individual.

The male organs consist of two *testes,* with their ducts, the *vasa deferentia,* and a *seminal vesicle.* In some flukes there are in addition a well-developed *cirrus,* or *penis,* and a *cirrus pouch;* these are lacking in *Clonorchis.*

The *testes* are a pair of much-branched organs lying in the posterior fourth of the body, one behind the other, and extending laterally beyond the rami of the alimentary canal. How many lobes has each?

The *vasa deferentia* (singular, *vas deferens*) arise near the middle of each testis and run forward to about the middle of the body, where they unite and, continuing forward over the loops of the uterus, open into the *seminal vesicle.* The wide seminal vesicle curves ventrally along the right side of the ventral sucker and unites with the terminal portion of the uterus to form a short common genital duct opening through the *genital pore* already noted as lying close to the median anterior margin of the ventral sucker.

The female organs consist of the *ovary,* the *vitellaria* or *yolk* glands, the *Mehlis gland,* the *seminal receptacle,* the *Laurer's canal* and the *uterus.*

The *seminal receptacle* is a conspicuous, lightly staining ovoid body just anterior to the first testis. It lies somewhat obliquely to the longitudinal axis of the fluke, with its left end the more anterior.

The *ovary* is a lobed organ lying in the median line just anterior to the seminal receptacle. It stains more deeply than the latter but is somewhat concealed by other parts.

The *vitellaria* lie exterior to the two intestinal rami and extend approximately from the region of the ventral sucker to that of the seminal receptacle. They are made up of numerous rounded glands connected by tubules and ultimately discharging through the paired *vitelline ducts.* These originate near the posterior ends of their respective vitellaria and curve to the middle line, where they are united on the dorsal side of the ovary. At the point of union the common duct expands somewhat to form the so-called *yolk reservoir.*

The *oviduct* is a tube which originates from the dorsal surface of the ovary and unites with the yolk reservoir.

Laurer's canal is a sharply defined sinuous tube which originates from the oviduct just before its fusion with the yolk reservoir and runs posteriorly around the left end of the seminal receptacle, to open through a dorsal pore near the center of the anterior testis. Its significance and function are matters of much dispute.

The *Mehlis gland*, formerly regarded as a shell gland, surrounds the junction of the oviduct and the yolk reservoir. It is in reality an aggregation of unicellular glands.

The *uterus* is a much convoluted tube packed with eggs, occupying the whole median portion of the body between the ovary and the ventral sucker, within the rami of the intestine. It opens through the genital pore.

The *egg* is dark brown, ovoid, and, like most fluke eggs, possesses a cap, or operculum, through which the embryo escapes. Is the opposite pole evenly rounded?

Excretory System.—Running forward from the excretory pore is the main duct of the excretory system, appearing as a clear median tube which extends forward to the region of the seminal receptacle where it bifurcates and is continued forward as a slender tube on either side to the anterior region of the worm. The finer tubules and their ultimate endings as the so-called *flame cells* cannot be seen in these preparations.

Nervous System.—The paired *cerebral ganglia* can be seen as somewhat stellate, more deeply stained spots laterad and dorsal to the middle portion of the œsophagus. Indications of the origin of the anterior and posterior nerves can be seen in favorable specimens.

The *circulatory* and *respiratory systems* are not present in the Trematoda.

OTHER SPECIES

Frog lung flukes belonging to the genus *Pneumonœces* are readily available and should be compared with *Clonorchis.* In the lack of the latter they may even be used as a type. Note particularly the feeble development of the ventral sucker, the shape of the testes and ovary, the distribution of the vitellaria, and the extent of the uterus.

The Liver Fluke of the Sheep.—This species, *Fasciola hepatica*, which is so widely used as a type in courses in general zoölogy, should now be examined in the light of the above studies. Note the very great development of the branches, or cæca, of the alimentary canal, the extensively branched testes, the smaller ovary and restricted uterus, and the position of the genital pore.

A blood fluke, *Schistosoma hæmatobium,* will be demonstrated as an example of a fluke in which the sexes are separate.

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CHAPTER III

THE LIFE CYCLE OF A TYPICAL TREMATODE

TECHNICAL SUGGESTIONS

For the study of the characteristic operculate eggs, the most certain source of supply is in the various species of frog trematodes. Permanent mounts, in balsam, are convenient.

Living miracidia are readily obtained from the eggs of *Diplodiscus* from the rectum, or of various *Gorgoderinæ* from the bladder of frogs. Placed in water at room temperature they will hatch overnight. If wanted more promptly, the miracidia will emerge within a half hour or so if placed in water heated to 35 to 40°C. For permanent mounts they may be collected in a watch glass with a minimum of water, killed in hot saturated corrosive sublimate solution, stained in borax carmine, and mounted in balsam in the usual manner.

For the study of the larval stages, snails should be collected from different localities, as considerable variation in intensity of infection as well as in species occurs. The best sources of material may be determined in advance of laboratory work by sorting as to species the snails from different localities and placing a half dozen specimens of each group in wide-mouthed 8-ounce bottles one-third full of water. Kept in this manner overnight the cercariæ escape and the intensity of infestation of the various groups can be determined by examining the water. Infected snails may be kept alive and will continue to discharge cercariæ for months if the water is changed frequently and lettuce is provided for food.

Permanent balsam mounts may be made after fixing in hot corrosive sublimate or in one of the picro-formal fixatives.

THE LIFE HISTORY OF THE SHEEP LIVER FLUKE

Preparatory to the practical work the student should review the life cycle of the sheep liver fluke, a form very generally discussed in beginning courses in zoölogy.

The adult fluke in the bile ducts of the liver deposits eggs which pass into the intestine of the host and out with the droppings. These eggs are operculate and develop the ciliated embryo, or *miracidium*. After two or three weeks in water the miracidium escapes and bores into the pulmonary chamber of a particular species of snail where it transforms into a *sporocyst*, an irregular mass without cilia and without a digestive tube.

Within these are developed numerous elongate *rediæ*, with simple, unbranched alimentary canal. These rediæ leave the sporocyst and invade the liver of the snail where they give rise to *daughter rediæ* or directly to the third form, or *cercariæ*. These are minute, tadpole-like forms with a bifurcate intestine, a tail, and, in this species, two suckers. The cercariæ escape from the snail and, losing their tail, become encysted on grass and other herbage.

PRACTICAL WORK

While *Fasciola hepatica*, the sheep liver fluke, occurs only in limited areas in the United States, most of our vertebrate animals, from fish, frogs, and reptiles to man, are infested by their own special species of flukes which undergo essentially the same cycle of development as does the better known species from the sheep. The larval stages of these flukes are to be found in snails from our ponds and lakes, sometimes two or more species together. Study and make drawings of the following stages:

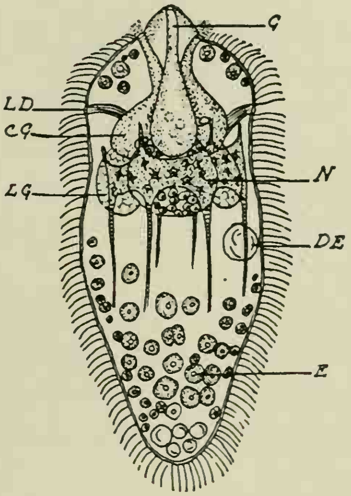

FIG. 4.—Miracidium of *Schistosomum japanicum*. *CG*, cephalic gland; *DE*, degenerate embryo; *E*, developing embryo; *G*, gut; *LG*, lateral gland duct; *N*, nerve center. (*After Faust and Meleney.*)

Eggs.—These may be studied in prepared slides or by obtaining living flukes from the lungs or intestines of a frog, teasing them apart, and mounting them in water. The eggs are ovoid bodies with a smooth brownish shell and a minute cap, or operculum, at one end (Fig. 3). The contained protoplasm may be undivided or in varying degrees of segmentation depending on the species. When the embryo develops, it pushes open the operculum and escapes as a free-swimming miracidium.

The *miracidium* (Fig. 4), is more or less conical, with a short papilla at its anterior end. The surface is covered by cilia giving the living organism a superficial resemblance to a rapidly moving infusorian. An eye spot is usually to be seen a short distance from the anterior end. A deeply staining mass posterior to the middle of the body is made up of the germ cells. The miracidia enter the pulmonary chamber of a preferred species of snail and transform into sporocysts.

By use of the appended key (p. 18) determine the genus of the living snails furnished for this exercise. This done, carefully snip away the shell and remove the snail to a dish of water. Note the large respiratory chamber on the dorsal surface and the very large yellowish or brownish liver which forms a large part of the coiled portion of the snail.

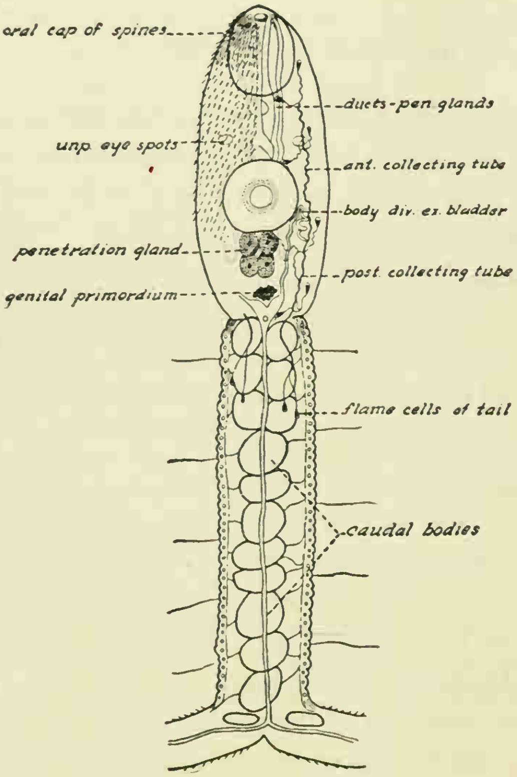
oral cap of spines
ducts-pen glands
unp. eye spots
ant. collecting tube
body div. ex. bladder
penetration gland
post. collecting tube
genital primordium
flame cells of tail
caudal bodies

FIG. 5.—Semi-diagramatic drawing of *Cercaria longifurca*. The body spination is shown only on the left side and the excretory system on the right. (*After Cort and Brooks.*)

At this stage there may be seen one or more clear colorless worms about half an inch in length, with well-developed setæ, or bristles. These have no relation to the developmental stages of the fluke but are commensal annelid worms of the genus *Chætogaster*.

Open and examine the pulmonary chamber of your snail. In a high percentage of specimens will be found the early larval stages of some fluke.

The *sporocyst* is an elongated sac with an outer cuticle, a thin muscular layer, and an inner epithelial layer. In the interior are groups of cells of varying size from which develop the next generation, the rediæ.

The *rediæ* are characterized by an unbranched saclike alimentary canal with a single opening, the mouth. The rediæ wander about in the snail, being especially abundant in the liver. Within them may be produced *daughter rediæ* or, especially in the fall and winter, the next larval form, the cercariæ.

The *cercariæ* (Fig. 5) are usually tadpole-like in shape and are at first very active. They possess a tail, one or two suckers, a bifurcate alimentary canal with a single opening, the mouth, and the rudimentary genital organs. The excretory system, which consists of a complicated series of "flame cells" and of collecting tubules, will be demonstrated (see p. 16). Its development in one of the holostome cercaria is shown in the accompanying figure from Cort and Brooks, 1928. In any locality there may be found cercariæ of several types which should be determined by the appended key. Occasionally while being studied, a cercaria may be seen to contract, form a clear colorless cyst about its body, and discard its tail, which may continue to thrash about independently for a time. The encysted forms are known as metacercariæ.

The encysted *metacercariæ* occur on vegetation or on, or in, other animals, depending on the species of fluke from which they are derived. Various crustaceans and insects, fish, and frogs are commonly infected and are the intermediaries through which the parasite is passed on to its final host.

Certain flukes, such as the schistosomes, or blood flukes of man and animals, omit the redia stage in their life cycle, cercariæ being produced within the sporocysts. Their characteristic fork-tailed cercariæ do not become encysted but bore actively into the skin of the final host. There will be demonstrated *Cercaria elvæ,* a species which is parasitic on some of the lower mammals and is often a cause of transitory skin irritation of man, known as "swimmer's itch."

KEY TO THE CHIEF GROUPS OF CERCARIÆ

Adapted from Lühe, 1909

A. Body of the cercariæ with strong longitudinal projections along the side. . . . . *Lophocercariæ*

AA. Body without such longitudinal projections.

  B. Two long, symmetrical projections from the end of, and several times exceeding the length of the body. Mouth opening in the middle of the ventral surface. Intestine simple, sac-like. . . . . . . *Gasterostome cercariæ*

  BB. Tail variously formed but never split to the base. Mouth at anterior end. Intestine forked.

    C. Ventral sucker lacking. . . . . . *Monostome cercariæ*

    CC. Ventral sucker present.

      D. Ventral sucker at the very end of the body directly before the base of the slender tail. . . . . *Amphistome cercariæ*

      DD. Ventral sucker in front of the caudal end of the body. *Distome cercariæ*

KEY TO THE MAJOR GENERA OF FRESH-WATER SNAILS

A. Shell spiral dextral (*i.e.,* with aperture to right of the axis when held apex uppermost with aperture facing you) Spire more or less elongate. . . . . Genus *Lymnœa*

B. Shell discoidal, usually sinistral. . . . . Genus *Planorbis*

C. Shell spiral, sinistral. . . . . Genus *Physa*

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CHAPTER IV

THE STRUCTURE AND LIFE HISTORY OF A TAPEWORM, TÆNIA PISIFORMIS (BLOCH 1780)

TECHNICAL SUGGESTIONS

The cosmopolitan *Tænia pisiformis* (*T. serrata*) of dogs is the most available material for this practicum. For the morphological study, species of *Multiceps* or any of the other tænioid cestodes of the dog are equally suitable. Proglottides of *Tænia saginata* of man are occasionally available from hospitals or may be purchased from biological supply houses.

Prepared slides of entire specimens of *Tænia pisiformis* are instructive, but in general it is more satisfactory to have separate mounts of developing, mature, and ripe proglottides and of the scolex. Directions for the preparation of these will be found in the Appendix, page 101.

The hexacanth embryos are more readily studied in eggs of the Hymenolepid tapeworms. Rats and mice, including laboratory varieties, are commonly infested by these worms, as are also chickens. At most seasons of the year viscera of freshly killed poultry can be secured from butcher shops and will yield ample material.

Cystic stages may be found in wild rabbits the country over, but in order to insure a supply of suitable material, eggs from ripe proglottides of *Tænia pisiformis* should be fed to tame rabbits some two months before needed.

If there are facilities for experimental work, cysts of this age may be fed to a dog at the time of the laboratory practicum and the adult worms may be recovered in about six weeks.

In lieu of rabbit material, cysts of *Tænia tæniaformis* of cats may be found in the liver of a high percentage of wild rats and mice. These are readily developed in kittens.

CHARACTERISTICS OF THE CLASS CESTODA

The **Cestoda**, or tapeworms, are flatworms which have, typically, the form of a ribbon made up of a large number of segments, or *proglottides* (singular, *proglottis*). At one extremity is the organ of attachment, the *scolex*, provided with suckers of varying form and number, and, in some cases, with hooks. The body cavity is lacking, as is also the digestive tract. Food is absorbed directly through the integument. With rare exceptions, the life cycles of tapeworms involve at least two hosts of different species.

The class **Cestoda** is divided into two subclasses, the **Monozoa** and the **Merozoa**. The former contains the most primitive Cestoda, unsegmented forms which are of special interest as showing relationships to the Trematoda, or flukes. Most of the described species are from fish.

The typical tapeworms belong to the subclass Merozoa. We shall take as a type *Tœnia pisiformis*, one of the most common tapeworms of the dog.

PRACTICAL WORK

Gross Examination.—A fresh or prepared specimen of *Tœnia pisiformis* will be furnished for examination. Note the length, the shape of body, the *head* or *scolex*, the *neck*, and the *strobila*, or chain of *proglottides*, or segments. About the middle of one side of the larger proglottides note the *genital pore*. Do these occur on the same side of all the proglottides? Count the number of proglottides. Make an outline drawing illustrating these points, indicating by dotted lines regions only partially represented.

Detailed Study.—Using stained and mounted preparations, study carefully the following points, making enlarged drawings of each region. As the specimens are thick and more or less opaque, it is important carefully to regulate the lighting and to use great caution to avoid injuring them.

Scolex.—Note the shape and relative size of the scolex. It is provided with four suckers for attachment to the host. In addition, *Tœnia pisiformis* is "armed" with a double circlet of attachment hooks, borne upon a slightly projecting *rostellum*. The hooks vary in number, size, and shape in different species of tapeworms and should be carefully studied in the specimens available. Examine several slides giving different aspects of those structures. Indicate also in your chart the short, unsegmented neck.

Developing Proglottides.—Examine proglottides from the first sixth of the body length and note the early traces of the reproductive organs. It should be recalled that the Cestoda are hermaphroditic. Two more deeply staining lines running in from the genital pore represent the *vas deferens* and the *vagina*. In the mesal line, near the lower portion of the proglottis, the vagina bends down to an indistinctly outlined mass, which is the developing ovaries. Passing forward from this is the unbranched uterus. The flocculent mass largely filling the proglottis is made up of the lobes of the testes.

Mature Proglottides.—Selecting a proglottis somewhat beyond the middle of the strobila, study the mature reproductive system. Compare Fig. 6, illustrating a related genus.

The more anterior duct passing mesad from the genital pore is the *vas deferens.* The racemose lobes of the *testes* are attached to the *vas deferens* by very delicate efferent ducts. They occupy what is generally interpreted as the dorsal surface of the segment. At its outer extremity the *vas deferens* enters the swollen *cirrus pouch,* within which it terminates in the *cirrus* or *penis.*

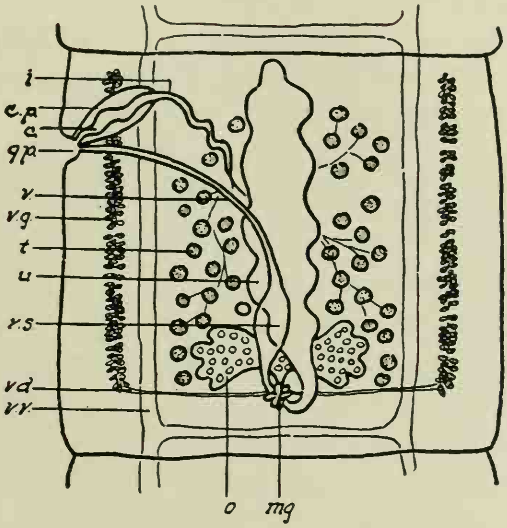

FIG. 6.—Mature proglottis of a tænioid tapeworm *Ichthyotœnia.* *c,* cirrus; *c.p.,* cirrus pouch; *g.p.,* genital pore; *i,* vas deferens; *m.g.,* Mehlis gland; *o,* ovary; *r.s.,* receptaculum seminis; *t,* testicle; *u,* uterus; *v,* vagina; *v.d.,* vitelline duct; *v.g.,* vitelline gland; *v. v.,* ventral excretory vessel. (*After Fuhrmann.*)

Running parallel and caudad to the *vas deferens* is the *vagina.* Towards the middle of the proglottis the vagina bends caudad and unites with the *oviduct* passing from the paired ovaries. Near its point of union the vagina enlarges slightly to form a *receptaculum seminis.* The ovaries, lying to either side of the mesal line, are of unequal size and are composed of a number of tubular follicles. In the middle line just back of the ducts from the ovaries is the rounded *Mehlis gland,* or so-called *shell gland.* Close to the caudal margin of the proglottis is the yolk, or *vitelline gland.* The vitelline duct runs forward from this to the *Mehlis gland.* From this point the *uterus* passes forward as an unbranched tube in the mesal line, extending to the anterior end of the proglottis. The female organs are confined to the ventral surface of the proglottides.

At either side of the proglottis may be seen two clear lines. The inner, larger of these represents the main canal of the *excretory system.* Close to the posterior margin of each proglottis is a transverse canal connecting the longitudinal canals. A second, much smaller longitudinal canal lies entad of the above described and may be seen in sections.

Outside of the excretory canal is the principal longitudinal nerve, on each side. Smaller nerves are present but cannot be seen in whole mounts.

The excretory and the nervous system are continuous throughout the length of the strobila.

Ripe Proglottides.—In the terminal segments the generative organs have been in large part supplanted by the uterus, which has become much branched and is filled with eggs already containing the six-hooked or *hexacanth* embryo. The number and form of the branches of the uterus are points of importance in classification, there being eight to ten irregular ramifications on each side in *Tœnia pisiformis.*

Examination of Sections.—Cross-sections through a mature proglottis emphasize the lack of alimentary canal and of body cavity, a condition typical of the Cestoda. The outermost layer of the body is a thick resistant *cuticle,* which is secreted by prominent, deeply staining, fusiform matrix cells embedded in the *parenchyma,* or tissue which fills all the space between the different organs and muscles. Immediately under the cuticle is a delicate layer of *circular muscles,* followed by a layer of *longitudinal muscles* whose cut ends can be seen among the matrix cells at their outer end. A second and much more extensive set of longitudinal muscles lies in the parenchyma entad of the matrix cells. Within these are the *transverse muscles* which run across the proglottis. Scattered through the parenchyma are the light-refracting, concentrically striated calcarious bodies which give to the worm its opaque white color.

The large pore near the lateral margin of the section is the *external excretory vessel* in cross-section. Immediately entad of this is the smaller *internal excretory vessel.* Lying just outside of the external excretory vessel is the large longitudinal nerve. Two smaller nerves accompany this but are hardly distinguishable in the sections.

Occupying the central portions of the section, between the excretory vessels of the two sides, are sections of the reproductive organs. In the sections furnished the major portions of this mass consist of lobes of the testes.

Life History.—Examine eggs from a ripe proglottis. These are best studied in fresh material, even if another species is the only one available; but prepared slides may be used

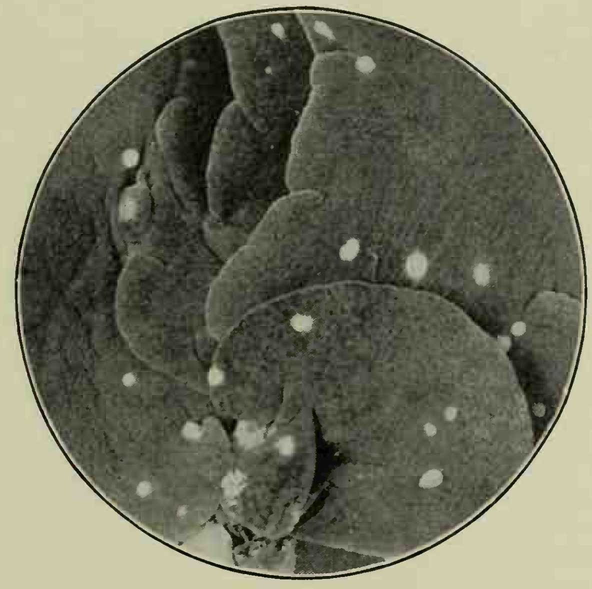

FIG. 7.—Cysts of *Tænia pisiformis* in the liver of a rabbit 24 days after experimental feeding. (*Orig.*)

It will be found that in the eggs ready to be discharged from the body, the six-hooked or *hexacanth embryo* is already formed. In the fresh material movements of the embryo can be readily studied. It should be noted that the so-called shell of eggs of tapeworms of the group under consideration is in reality an embryonic membrane, the embryophore, formed from cells of the egg itself. In *Tænia pisiformis* and related forms, this embryophore is finely striated, appearing to be made up of many fine rods placed side by side. The hexacanth embryo is also commonly known as the oncosphere.

Hatching of the Eggs.—When the eggs of the tapeworm are taken up by an appropriate animal, the embryo is liberated by the action of the digestive juices. In the case of *Tænia pisiformis* the intermediate host is the rabbit, and it should be understood that while the general features of the life cycle are typical for the group, the details apply to this species only.

Development within the Liver.—The liberated embryos, by means of their armature of hooks, penetrate the walls of the intestine and are carried by the blood current to the liver. There they lose their hooks and by the sixth day they have enlarged sufficiently to appear as little transparent vesicles (Fig. 7). By the twelfth day, having attained a size of 3 mm., they present the appearance shown in the slides provided, that of little whitish bodies made up of a very loose parenchyma tissue, limited by a delicate cuticle. The head of the future tapeworm begins to form at this stage.

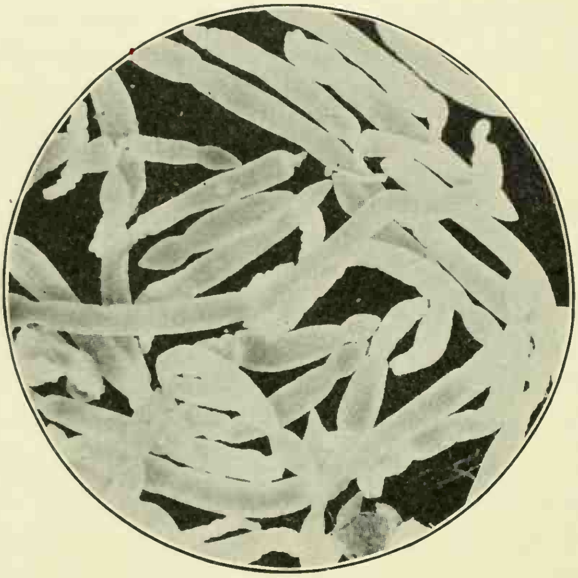

FIG. 8.—Migratory larvæ of *Tænia pisiformis* as they may be found free in the body cavity of the rabbit 5 weeks after infection. (*Orig.*)

Development in the Mesenteries.—About a month after infestation the very active larva squirms out from the liver and lives free in the body cavity of the rabbit for several days (Fig. 8). The central part of the larva degenerates and becomes filled with a fluid, and the rounded mass attaches to the peritoneum or mesenteries of its host as a *cysticercus.* This *cysticercus,* with its invaginated head of the future tapeworm, at the end of a few weeks is about the size of a pea, and is ready to transfer to the definitive host and there develop into the mature tapeworm. This process requires about two months before ripe segments are ready to be discharged.

Sketch a bit of mesentery of an infested rabbit, showing the cysts of *Tœnia pisiformis, in situ.* Make careful drawings of mounted cysts, showing the extruded larva.

References

The discussion of a tænioid tapeworm of man, in any good textbook of parasitology, will be broadly applicable to the species here used. In addition the following references may be consulted:

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HALL, M. C., 1919. The adult tænioid cestodes of dogs and cats, and of related carnivores in North America. *Proc. U. S. Nat. Museum,* **55**: 1–94.

MONIEZ, R., 1880. "Essai monographique sur les cysticerques," 190 pp., 3 plates. These, Lille.

CHAPTER V

COMPARATIVE STUDY OF SOME IMPORTANT CESTODES

TECHNICAL SUGGESTIONS

Mature and ripe proglottides of *Tænia saginata* of man are readily obtainable. The former is so similar to that of *Tænia pisiformis* that it is not necessary to devote time to its study, but the ripe proglottides should be available for comparison with those of *Tænia solium*, which can be purchased from dealers. Demonstration specimens of the cysticeri, entire and in section in beef or pork, should also be purchased.

Scolices of these worms are rarely available, but the everted heads from the cysticerci of the two species are more readily obtainable.

Hymenolepis nana and *Hymenolepis diminuta* are commonly found in both wild and laboratory rats and mice. Whole mounts should be made for morphological study. The cysticeroids of *H. nana* may be obtained by feeding the eggs in numbers in bread and milk to rats and sectioning the intestine 24 to 48 hours later. *H. diminuta* developmental stages may be obtained most readily by feeding eggs to adult meal beetles, *Tenebrio molitor*.

Dipylidium caninum and closely related species are very common in pet dogs and cats. Their characteristic egg packets in fecal material should be preserved in 10 per cent formalin. Cysticeroids develop in larval fleas and may be found in a small percentage of the adult insects from infected dogs.

Diphyllobothrium latum is endemic in some sections of this country; but, in general, reliance must be placed on dealers. If living specimens are available, the eggs should be cultured in water at summer temperatures and the ciliated embryos will escape in 10 to 12 days.

Multiceps serialis is a widely distributed parasite of dogs. The cystic state of this parasite is often noted in the muscles of rabbits. Sections of such cysts should be made to show the multiple invaginated heads. Experimental feedings of dogs are readily carried out, if there are facilities for keeping the animals under control.

Echinococcus cysts are most readily obtained from slaughter houses in our Southern states. Sections of the cyst wall and mounts of fragments of the germinal layer showing larval scolices should be made or purchased. Strangely enough, the minute adult worms have been very rarely found in dogs in this country. They should be sought in examination of these animals in the South, due regard being taken of the danger to humans from careless handling of infective material.

PRACTICAL WORK

The purpose of this practicum is to become acquainted with the principal tapeworms affecting man and some of the animals with which he is closely associated. Using your study of *Tænia pisiformis* as a basis, carefully examine such of the following species as are available, making drawings of significant features.

Tænia solium *L.*, the pork tapeworm of man, develops as a cysticercus or "bladderworm" in the flesh of hogs. The adult worm in man measures 2 to 4 meters or more in length and has 300 to 1,000 proglottides. The head bears a short rostellum with a double crown of hooks. The ripe proglottides are usually discharged from the human host in short chains and since the species is a dangerous one it is important to note that the uterus of these ripe segments is characteristically branched in a dendritic (treelike) manner with only 5 to 10 branches on each side. The eggs are globular, about 50μ in diameter, with a thick, radially striated embryophore ("shell").

Tænia saginata (Goeze), the beef tapeworm, undergoes its larval stage in beef. It is the commonest of the large tapeworms of man in the United States and in most parts of the world. It may measure 12 meters or more in length and possess as high as 2,000 proglottides. The head has four suckers but is unarmed, *i.e.*, it lacks hooks. In the ripe segments the uterine branches are more numerous (15 to 30) than in *T. solium* and are dichotomous. The eggs closely resemble those of *T. solium* but average slightly larger.

Compare mature proglottides of *T. solium* and *T. saginata* with those of *T. pisiformis* and carefully compare the uterine branching in the ripe segments of the three species.

Examine demonstration specimens of the cysts in pork or beef, if available. The characteristics of the heads of the two species may be seen in everted heads of the larvæ.

Hymenolepis diminuta (Rudolphi) is a rat and mouse tapeworm that is also transmissible to man. It is a slender worm 20 to 40 cm. in length and 3.5 mm. at its greatest breadth. The head is unarmed and has four suckers. The segments, which may number upwards of 1,000, are very short and have the genital pores all on the same side. In each mature segment there are three large round testes, in transverse order. Between the first and second of these are the paired, branched ovaries and the vitelline gland. The sac-like uterus completely fills the ripe proglottides. The eggs are large and transparent with two envelopes, the inner double, and with two polar knotlike projections. The larval stages develop in various cereal-infesting insects. (Fig. 9).

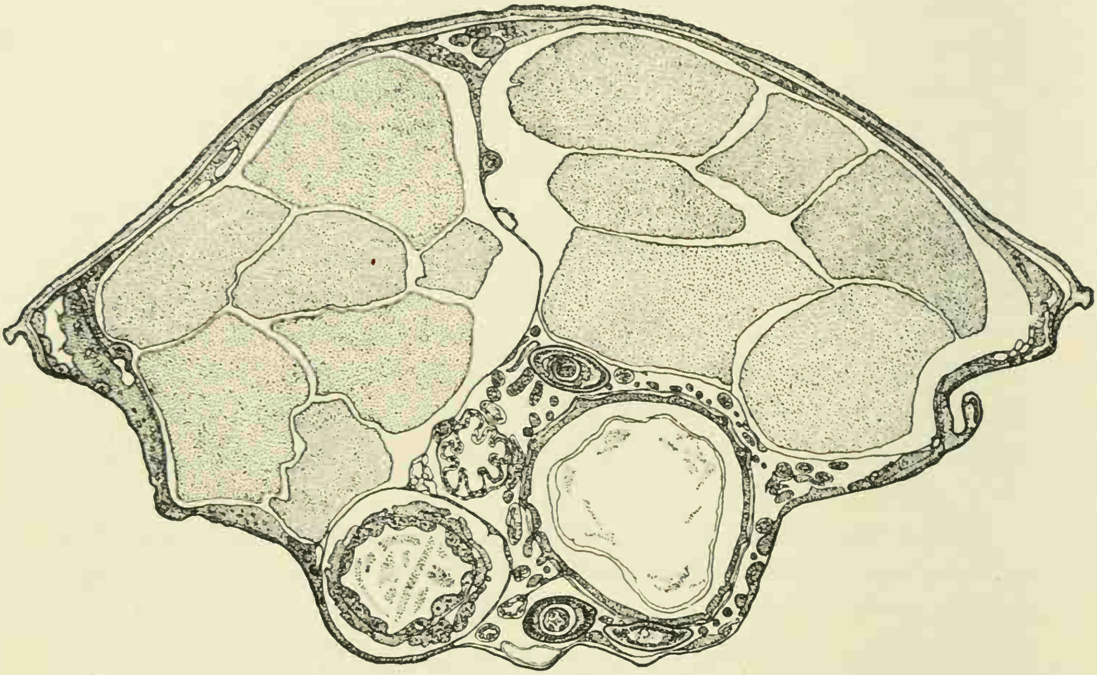

FIG. 9.—Cysticercoids of *Hymenolepis diminuta* in body cavity of a beetle, *Tenebrio molitor*. The three specimens lie adjacent to the large section of the alimentary canal.

Hymenolepis nana (von Siebold) is the dwarf tapeworm of man, a species so minute and threadlike that it is usually overlooked in macroscopic examinations. The adult worms measure from 10 to 25 mm. (1 inch or less). The head has four suckers and a simple crown of hooks. The general morphology is similar to that of *H. diminuta*. The eggs can be distinguished by the fact that there are filiform projections from the knobs of the inner membrane. A striking exception to the usual rule is the fact that these worms are able to complete their development in a single host.

Dipylidium caninum (Linn.) is one of the commonest tapeworms of pet dogs and is also capable of developing in man. It measures 10 to 40 cm. in length. The retractile rostellum is provided with three or four rows of minute, thornlike, easily detached hooks; the suckers are relatively large and ellipsoid. The mature proglottides somewhat resemble cucumber seeds in shape and are provided with a genital pore on each side and with double sets of reproductive organs. The uterus forms numerous diverticula, enclosing packets of 8 to 15 eggs. Distinguish in mature segments the genital pore on each side, with cirrus, cirrus pouch, vas deferens, vagina, ovary, vitelline glands, and the diverticula of the uterus. The cysticercoid stage develops in fleas and lice of the dog and cat.

Diphyllobothrium latum (Linn) (=*Bothriocephalus* of older writers) is the broad or fish tapeworm of man and various carnivores. It is the largest of the tapeworms of man, since it may attain a length of 15 meters or even more and possess from 3,000 to 4,000 proglottides. The ovoid head has two elongate attachment grooves, or bothridia. The proglottides are much broader than long and in their center is the uterus, in a rosette-like coil (Fig. 10). In the fresh specimen this appears as a dark median area which readily distinguishes the worm from the large tænias of man. The genital pore is situated in the mid-ventral line instead of laterally, and slightly farther back is a second opening through which the eggs are laid. The paired ovary is at the posterior end and the vitelline glands at the sides of the proglottides. The eggs are large, elliptical, brown, and provided with a cap, or operculum.

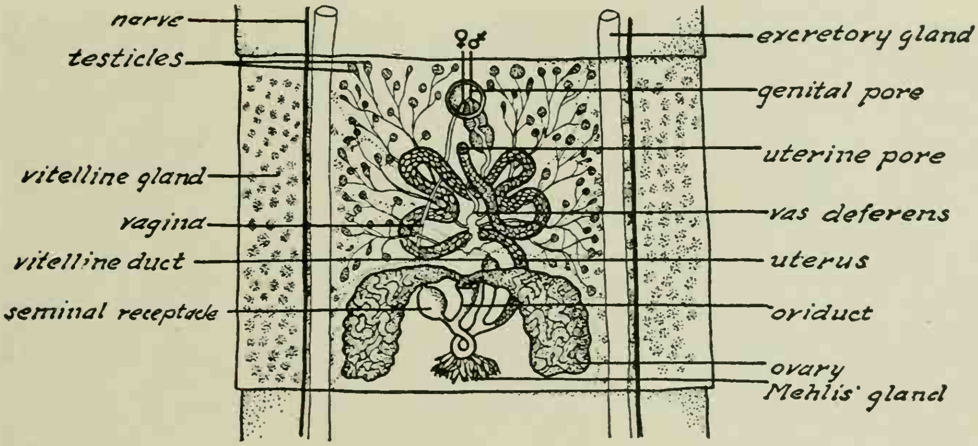

Fig. 10.—Diagram of a mature proglottis of *Diphyllobothrium.* (*After Verdun and Mandoul.*)

The fish tapeworm of man is one of the few cestodes known to require three hosts for its life cycle. From the egg there emerges a free-swimming ciliated embryo known as a *coracidium,* which after a short time enters one of various minute Copepod crustaceans. Here it develops into a first larval stage, and when the infected crustacean is devoured by pike, pickeral, burbot, and certain other fish, the larva develops into an elongate wormlike *plerocercoid* encysted in the muscles. In this stage it is infective to man.

Make sketches of a mature segment, a scolex, and eggs, showing the above features. Plerocercoids of this or related species will be demonstrated.

Multiceps serialis (Gervais) is a tapeworm of dogs which in its morphology closely resembles *Tænia pisiformis.* In its development in the intermediate host it presents a striking departure from the usual type. The cysts (Fig. 11), occur as large, so-called "boils" in the muscles of rabbits. They are filled with a limpid fluid, and on the interior are great numbers of minute granular bodies, each of which represents the invaginated head of a future tapeworm. In other words, worms of the genus *Multiceps* exhibit polyembryony, or the production of many individuals from a single egg. Examine demonstration specimens of the cysts *in situ,* and study prepared slides showing the invaginated scolices.

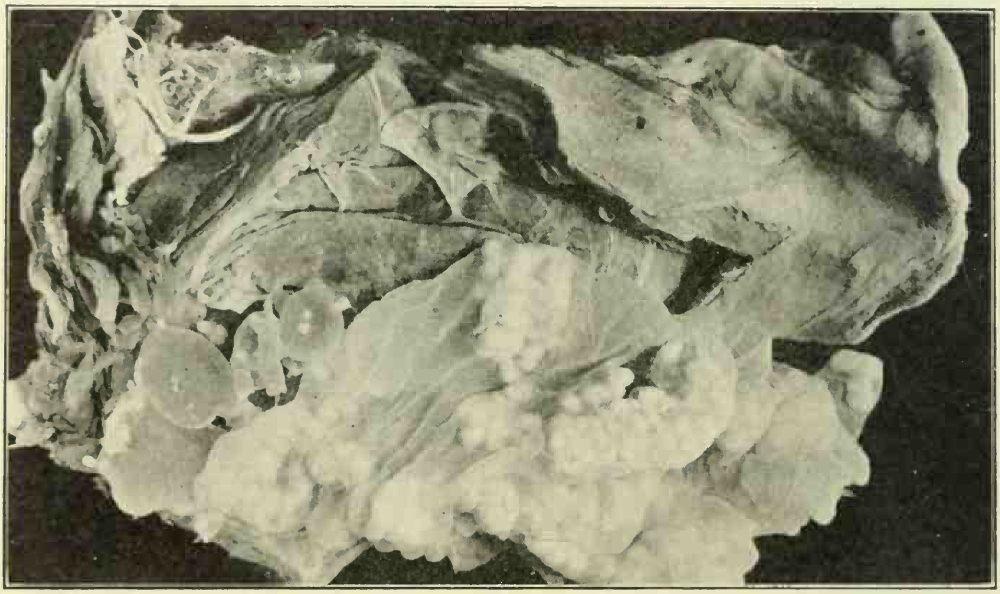

FIG. 11.—A cyst of *Multiceps serialis* from the shoulder of a jack-rabbit. Each of the rounded white bodies is a head capable of developing into a tapeworm if ingested by a dog. (*Orig.*)

Echinococcus granulosus (Goeze) is the dangerous hydatid tapeworm which develops its cystic stage in a wide range of mammals, including man. The adult is a very small worm living in the intestine of dogs. It measures a fourth of an inch or less in length and has but three proglottides. The head is very small, with a prominent rostellum armed with a double row of hooks. The terminal, ripe proglottis comprises about half the entire length of the worm but contains a relatively small number of eggs.

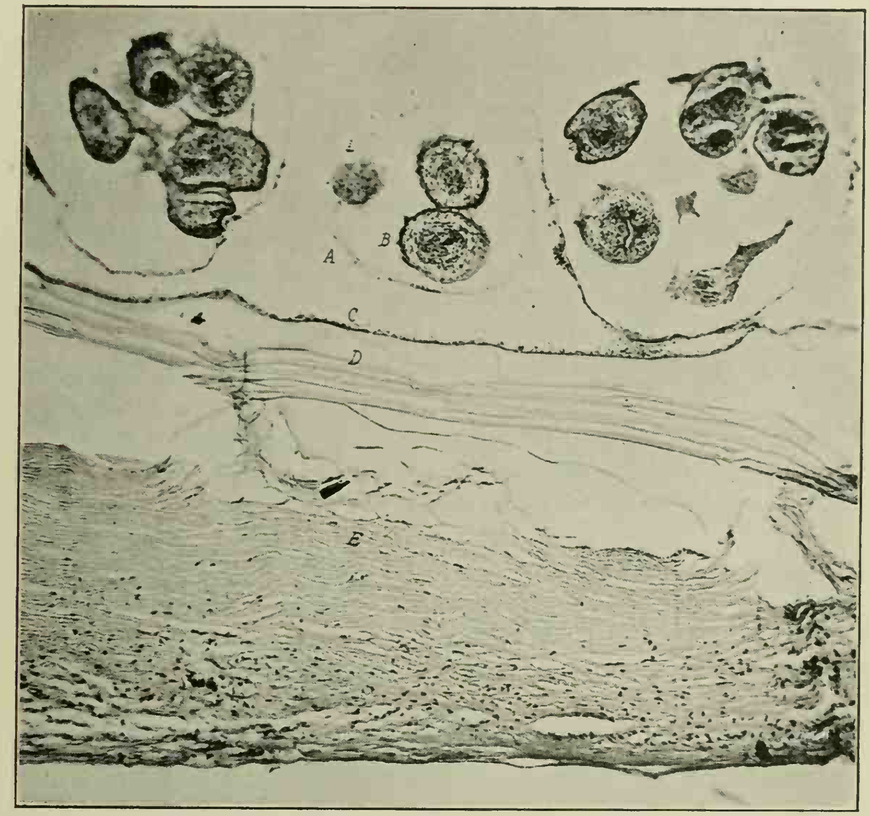

Fig. 12.—A section of the cyst of *Echinococcus granulosus* in the liver of a pig. *A*, brood capsule; *B*, scolex; *C*, germinal layer; *D*, cuticle; *E*, adventitious layer, formed by the host. The three layers have been separated in the process of sectioning. (*Orig.*)

The cysts develop slowly in the intermediate host but in man may attain the size of an orange or even of a human head. The thick wall of the cyst is composed of an outer adventitious layer formed by connective tissue of the host. Next is a firm lamellated layer called the cuticular membrane. Within this is the thin germinal layer. From the germinal layer and from certain scolices which undergo metamorphosis, there are formed many brood capsules within which arise numerous larval scolices. Thousands of these potential tapeworms may be present in a single hydatid cyst. The brood capsules within which they develop become detached from the germinal layer and float in the fluid as the so-called "daughter cysts."

Examine demonstration specimens of hydatid cysts. Study and make drawings of sections of cysts in liver or lung tissue of the intermediate host and of surface views of the germinal layer with developing scolices.

CHAPTER VI

ASCARIS LUMBRICOIDES AS A TYPE OF THE NEMATODA

TECHNICAL SUGGESTIONS

Specimens of the pig ascaris are to be obtained from slaughter houses and preserved for dissection in 10 per cent formalin. The larger horse ascaris makes an excellent object for laboratory dissection but is rather difficult to obtain.

Eggs should be removed from the terminal portions of the uteri of fresh specimens and cultured at summer temperature under a shallow layer of 2 per cent formalin to prevent contamination. All stages will be found for study in about two weeks.

Experimental animals—mice, rats, or guinea-pigs—may be fed cultures of infective larvæ on bread and the pathologic conditions in the lungs demonstrated a week later.

The migrating larvæ can most easily be recovered by chopping the liver and lungs and placing them in the simplified Baermann apparatus (p. 107). Then they will migrate out into the warm water and settle to the bottom.

For demonstrating the larvæ in the liver tissue, a heavily infected animal should be killed about the fourth day after the experimental feeding and small portions of the liver fixed in Zenker's fluid. For stages in the lung, it is better to fix in acetic-alcohol (glacial acetic, 1 part; absolute alcohol, 3 parts) some 10 days after infection. Paraffin sections of 10 to 15 μ in thickness should be cut, stained in Delafield's hæmatoxylin, and mounted in the usual manner.

For the infection experiments the dog ascaris, *Toxocara canis*, or *Toxocara mystax* from the cat will serve as well. Demonstrations of these common parasites should be shown and their eggs studied in comparison with those of *Ascaris lumbricoides*.

At this time there should also be demonstrated specimens of *Gordiacea* and of *Acanthocephala*, forms which can hardly be given special attention in an introductory course.

CHARACTERISTICS OF THE ASCAROIDEA

The class *Nematoda* contains species of great economic importance to animals and plants. Many of the species parasitic in animals pass part of their cycle free in the soil. The majority of the species of the group are oviparous, although some are viviparous. The larvæ undergo typically two molts before they are in the infective stage. As an introductory type *Ascaris* *lumbricoides* serves admirably, being large enough for easy dissection and presenting no complicated or highly specialized arrangement of organs.

Ascaris lumbricoides belongs to the superfamily *Ascaroidea.* In the course of the following study, the student should carefully check over the characteristics of this group, as given by Yorke and Maplestone: "Eunematoda; usually fairly large and stout; head bilobed; œsophagus frequently more or less enlarged posteriorly, but without a definite spherical posterior bulb containing a valvular apparatus (except in *Dujardinia,* where there is a small, unarmed bulb), with or without diverticula. Spicules equal or unequal. Females not much larger than the males."

PRACTICAL WORK

External Features.—Note the general form of the body, which in the Ascaridæ is relatively thick. The mouth is surrounded by three lips, one dorsal and two ventro-lateral. Running lengthwise of the body are four equidistant lines, a *dorsal,* a *ventral,* and two *lateral* lines. About two millimeters from the anterior end is the minute excretory pore in the median ventral line.

Dissection.—The female worm may be distinguished from the male by her greater size and gently curving caudal end. In the male the caudal end is sharply curved to form a hook, and two spicules may be seen at the anal opening. In the female the *genital opening* is also ventral and situated about one-third the length of the worm from the anterior end. The mouth opens between the three lips at the anterior end. The anal opening is at the caudal end.

Make an enlarged drawing of the ventral anterior end of the body showing the lateral lips and the position of the excretory pore. Make an outline drawing of the entire worm showing the position of the mouth, anus, excretory pore, genital opening, and lateral line.

Slit a female worm very carefully longitudinally just laterad of the median dorsal line and pin out the body in a dissecting pan. To prevent the specimen from drying, cover it with ½ inch of water. Note the alimentary canal consisting of a straight tube running from mouth to anus. Three sections may be distinguished: a short *œsophagus,* the long flattened *mid-intestine,* and a short *rectum.* The lateral line can be seen as an inward projecting ridge separating the longitudinal muscles. Locate the genital opening, or *vulva,* about one-third the length of the worm from the mouth, on the mid-ventral line. Passing in from this is the short *vagina,* which soon divides into two long tubes which are much convoluted and are filled with eggs. The portion of these tubes nearest the vagina represents the *uterus,* the middle portion is the *oviduct,* and the thinner distal portion the *ovary.* Enclosed in the ovary is the long central rod, or *rachis,* around which the eggs are arranged.

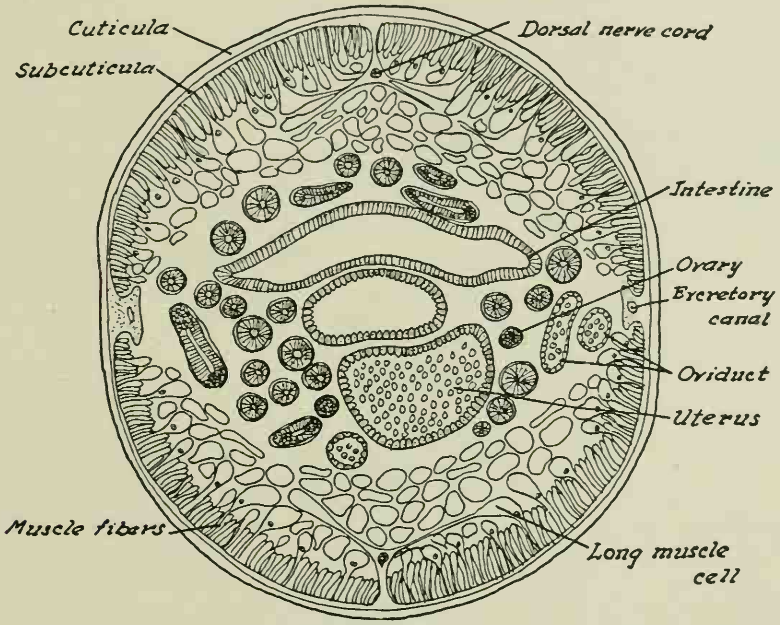

FIG. 13.—Cross-section through the mid-body region of a female *Ascaris megalocephala.* (*After Kükenthal.*)

Make a drawing showing the alimentary canal, the vagina, the uterus, the oviducts, and the ovary.

In a similar manner prepare a male specimen for study of the internal anatomy. The male reproductive organs are situated posteriorly. A long, single, much-coiled tube opens into the *cloaca,* a short portion of the alimentary tract between the rectum and anus. This tube is divided into three uneven portions: the *testis,* the longest portion, which is a thin convoluted tube containing a rachis with the sperm cells arranged around it similarly to the condition in the female; the *vas deferens,* the part of this tube not containing the rachis; and the *seminal vesicle,* the central portion, which is much shorter and thicker. The terminal portion is again thinner and quite short, having a musculature of its own. It serves as the ejaculatory duct.

Compare the male and female worms carefully, but drawing of male may be omitted.

Cross-sections.—Examine prepared slides of cross-sections of *Ascaris lumbricoides* or of a related species. Under low power make a drawing showing the body wall, the alimentary canal, reproductive organs, and the dorsal, ventral, and lateral lines.

Using high power, make a drawing showing the body wall: the cuticle with its various strata, the hypodermal layer from which the cuticle is a derivative, and the single layer of longitudinal muscles. Each muscle possesses a protoplasmic core with a nucleus, and a contractile sheath. If possible, find a section cut through the nucleus to include in your drawing. Each muscle cell, or fibril, is enclosed in a thin connective sheath the nuclei of which are often discernable.

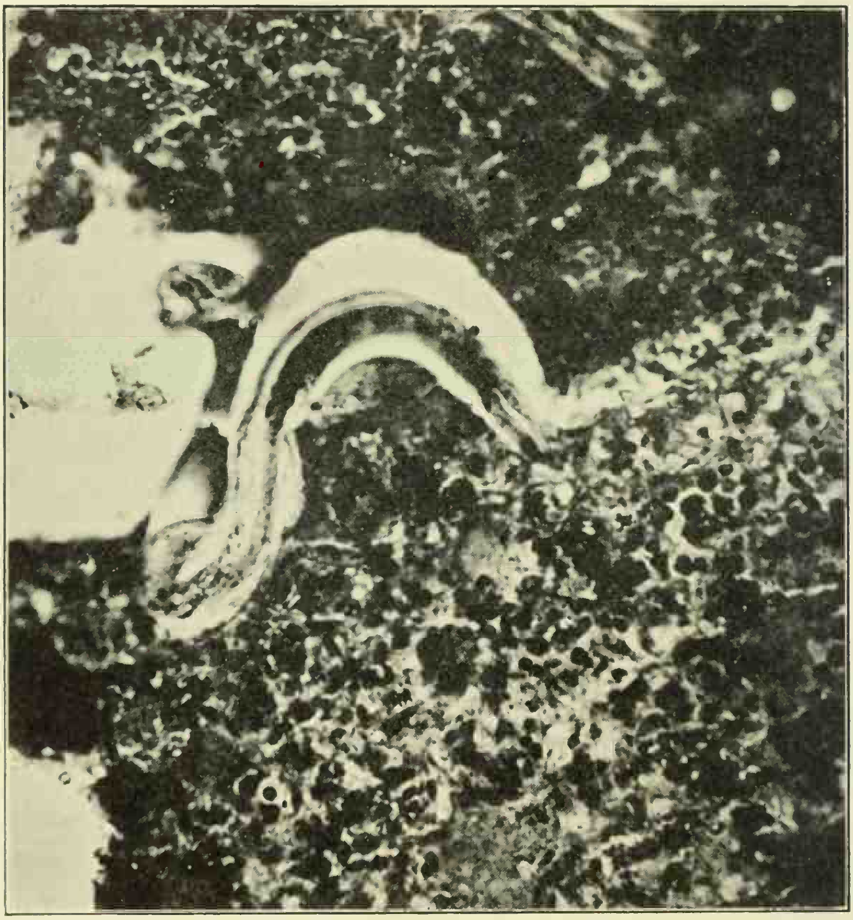

FIG. 14.—Migrating larva of *Ascaris lumbricoides* in liver of guinea-pig 6 days after feeding. (*Orig.*)

Development.—The eggs of *Ascaris lumbricoides*, as of many Eunematoda, develop directly, without the necessity of an intermediate host. In the course of development the lervæ undergo a migration through the liver and lungs of their new host, increasing in size and finally returning to the intestine to mature.

Using this species or the related *Toxocara* from the cat or dog, make a series of sketches of first, the unsegmented eggs from the uterus; and second, various segmentation stages from eggs which have been incubated at summer temperature for a few days. The eggs are not infective until they have reached the coiled embryo stage. In both *Ascaris lumbricoides* and *Toxocara canis* (*Belascaris marginata*) this requires upwards of 2 weeks.

Examine the demonstration specimens of Ascaris larvæ in the tissues of the liver (Fig. 14) and lungs of experimentally infected rats. If available, note the evidences of "Ascaris pneumonia" in an animal which has been fed infective eggs of the worm about a week previously.

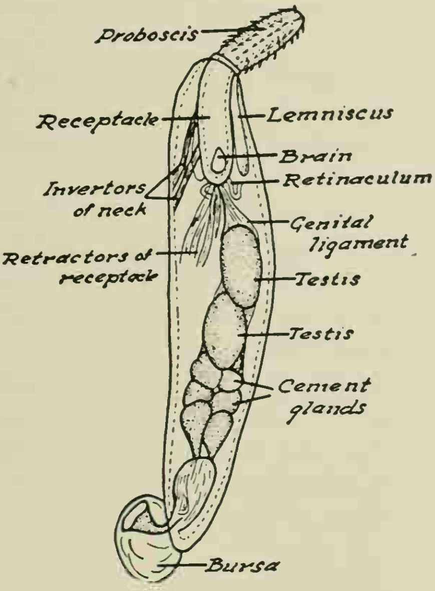

FIG. 15. — *Acanthocephalus ranæ* of frogs and salamanders. (*After Van Cleave.*)

Demonstrations of Gordiacea and of Acanthocephala.—Compare your specimen of Ascaris, a typical nematode, with the demonstrations of representatives of the Gordiacea and the Acanthocephala.

The *Gordiacea* are the so-called "hair-snakes." Their bodies are slender and wire-like and lack an alimentary canal in the adult stage. In this stage they are free-living, but in the larval stage they are parasitic in insects and less frequently in other invertebrates. As parasites of grasshoppers they often play a part in the natural control of these pests.

The *Acanthocephala* (Fig. 15) are characterized typically by the presence of a protractile proboscis, armed with numerous hooks, and by the lack of an alimentary canal. The larval stages are found in crustaceans, insects, fish, and small mammals. Adults are especially abundant in birds and fish. The demonstration specimens are of *Macrocanthorynchus* (*Echinorynchus*) *gigas*, a common parasite of hogs. Note also the demonstration of eggs, which are 90 to 100 μ long, nearly cylindrical, with a three-layered shell, the outer layer of which is marked by numerous depressions suggestive of those of an almond shell.

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Martin, H. M., 1926. Studies on the *Ascaris lumbricoides*. *Univ. Nebr. Agr. Exp. Sta., Research Bull.* 37, 78 pp.

May, H. G., 1919. Contributions to the life histories of *Gordius robustus* (Leidy) and *Paragordius varius* (Leidy). *Illinois Biol. Monographs*, 5 (2): 127–238.

Ransom, B. H., and W. D. Foster, 1920. Observations on the life history of *Ascaris lumbricoides*. *U. S. Dept. Agr., Bull.* 817, 47 pp.

Van Cleave, H. J., 1919. Acanthocephala from the Illinois River, with descriptions of species and a synopsis of the family Neoechinorhynchidæ. *Illinois State Natural History Survey Bull.*, 13 (8): 225–254.

CHAPTER VII

THE HOOKWORMS OF MAN AND ANIMALS

TECHNICAL SUGGESTIONS

Ancylostoma caninum affords the most satisfactory laboratory type for the study of the hookworms. In almost any section of this country a systematic examination of several freshly killed laboratory dogs and cats, or dogs and cats from the city pound will furnish abundant material. If necessary, specimens mounted or unmounted can be purchased from dealers.

The worms should be thoroughly shaken in a vial of clean water to remove mucus and debris from the mouth region, and then killed in hot 70 per cent alcohol plus 5 per cent of glycerine. When the alcohol has been allowed to evaporate, they may be mounted in glycerine jelly and sealed with Noyer's cement. Very satisfactory preparations may be made by passing from plain 70 per cent alcohol to lactophenol mixture in which the specimens are mounted. For temporary examination either lactophenol or 80 per cent carbolic acid in absolute alcohol may be used. Such temporary mounts are preferable for small classes with abundant material since they allow manipulating of .the specimen.

Eggs may be obtained in quantity by removing the rectal content of an infected dog or cat, sedimenting by several changes of water in a tall bottle, straining through a close-meshed sieve (*e.g.*, a tea strainer), and preserving in 10 per cent formalin. Random examination of feces of cats and dogs will often yield abundant material.

For larval stages cultures should be made by mixing fecal material containing eggs with equal parts of animal charcoal or heat-sterilized humus moistened with water and stirred to a paste. The moisture content being maintained, they are kept for a week in a warm room or in an incubator maintained at 25 to 30°C. and then isolated by means of the Baermann apparatus (see Appendix) or by the simple method of White, 1927. The culture is early placed in a Syracuse watch glass in a crystallizing dish with water equal to about half the depth of the watch glass, and the whole is covered by a large watch glass. The larvæ as they reach the infective stage wander from the culture into the water and may be recovered in numbers by pouring this off into a test-tube, in which they will settle to the bottom.

An instructive demonstration of the nematode population of the soil is afforded by placing a pint of rich garden soil in the Baermann apparatus for a few hours or overnight and draining the sediment into a large test-tube. A culture of infective hookworm larvæ should be included. Demonstrations of the larvæ in the skin may be purchased.

CHARACTERISTICS OF THE STRONGYLOIDEA

Among the most important nematode parasites of man and animals are the blood-sucking hookworms and related forms grouped in the superfamily *Strongyloidea*. They are of moderate size with an elongate, cylindrical, rarely filiform body. The males possess a caudal bell-shaped inflation known as the *bursa* which is supported by thickened rays and has two equal or subequal spicules. The œsophagus is more or less swollen posteriorly but never with a terminal bulb.

We shall study the common dog and cat hookworm, *Ancylostoma caninum*, as the most available example of the important hookworms.

PRACTICAL WORK

Ancylostoma caninum.—Compare the general form of the hookworm body with that of *Ascaris lumbricoides*. Note that the anterior end is bent dorsad and that it bears the wide mouth or buccal cavity. On the female locate the vaginal opening somewhat more than two-thirds of the length of the worm caudad, and the anal opening near the caudal end. In the male specimen study the characteristic caudal bursa and its supporting rays. How does the caudal end of the male Ascaris differ from this?

Selecting the specimen which shows most nearly a front rather than a profile view of the head capsule (Fig. 16), note the pair of strong, three-pronged teeth on the ventral wall of the capsule (remember that the head is bent dorsad and hence these teeth appear at the anterior end of the worm). Near the base of the capsule are the two broad, triangular, latero-ventral pharyngeal plates with their apices projecting into the pharynx. Between them is the dorsal pharyngeal tooth, a narrow elongate structure which appears rod-like in this view. Following the short bulbous pharynx is the elongate, cross-striated œsophageal bulb. The intestine passing from this point to the tip of the body is almost concealed by the coils of the reproductive organs. These in the female are the ovarian and the uterine tubes, the latter discharging the eggs through the vaginal opening in the posterior third of the worm. In the male the long coiled testes lying at the side of the intestine open into a broad sac, the seminal vesicle, about the middle of the body. From this the ejaculatory duct passes to the ventral side of the tip of the tail within the bursa. From this point extend the two long, threadlike spicules.

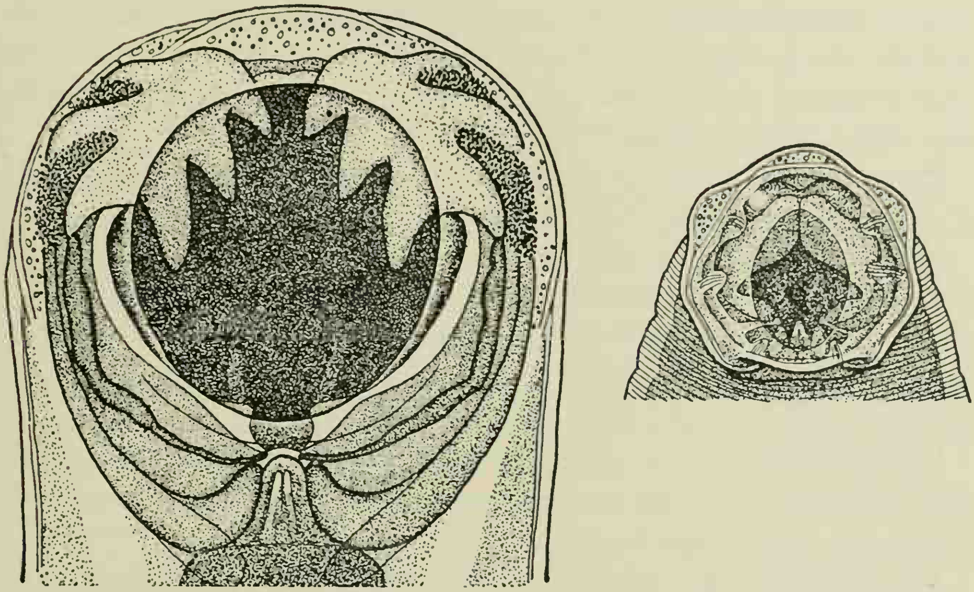

FIG. 16.—Head end of *Ancylostoma caninum* and of *Necator americanus* drawn to the same scale. (*After Looss, that of Necator reduced.*)

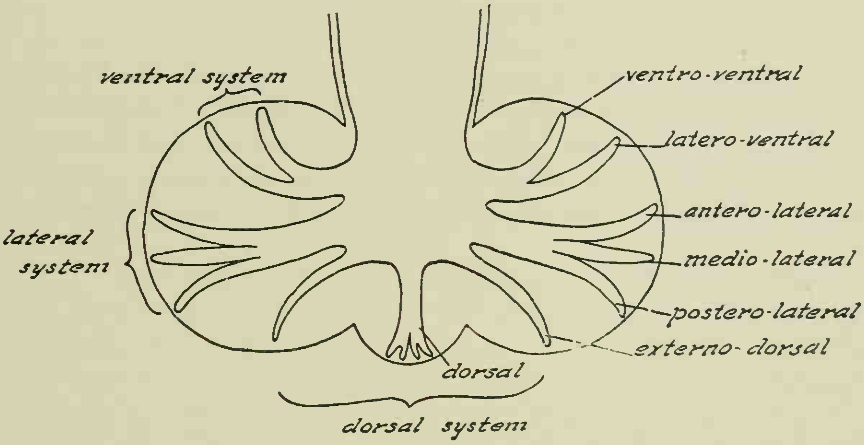
ventral system
ventro-ventral
latero-ventral
lateral system
antero-lateral
medio-lateral
postero-lateral
externo-dorsal
dorsal
dorsal system

FIG. 17.—Diagram of the caudal bursa of the male *Strongyloidea*. (*After Baylis.*)

The bursa of the male is a bell-shaped expansion of the cuticle supported by thickened rays which branch in a manner characteristic of the species. Compare carefully with the diagram (Fig. 17), identifying the various systems of rays.

Make a large drawing showing the general features above described.

Compare with the demonstration of the head capsule of *Ancylostoma duodenale* of man, noting especially differences in the paired teeth.

Study also the demonstration of the buccal capsule of *Necator americanus* (Fig. 16) and note the two chitinous plates which replace the ventral teeth found in the genus *Ancylostoma*. The latero-ventral pharyngeal plates and the dorsal pharyngeal tooth also differ in this genus.

Development.—Make drawings of various segmentation stages of the hookworm eggs from feces.

Make a careful study of the sheathed infective larva. If living material is available, note the movements and combine study of the living and the mounted worms. Make a drawing showing the buccal cavity, œsophagus, intestine, genital pore, genital rudiment, and anal opening of the larva.

Penetration of the Skin by Hookworm Larvæ.—Examine the demonstration showing a section of skin and of a larval hookworm which has penetrated its outer layers.

Soil Nematodes.—Examine a preparation of living nematodes, isolated from garden soil by the use of the Baermann apparatus, and distinguish the hookworm larvæ.

References

The standard textbooks on parasitology—Braun, Brumpt, Chandler, Faust—and such sources as the "Reference Handbook of Medical Science" afford easily accessible discussion of the morphology and biology of the hookworms of man. For references to more special papers, see the "Bibliography of Hookworm Diseases" issued by the International Health Board, 1922.

CHAPTER VIII

TRICHINELLA SPIRALIS AND RELATED FORMS

TECHNICAL SUGGESTIONS

The importance of *Trichinella spiralis* as a parasite of man and animals and the ease with which all stages of its life history may be demonstrated make it especially desirable for laboratory study. Other representatives of the group may be demonstrated. The characteristic eggs of Trichuris should be studied.

The most convenient sources of Trichinella for feeding experiments are wild rats from slaughter houses, particularly from local establishments. Here the infection may run as high as 75 per cent, though such high incidence is exceptional. Another convenient reservoir is the vagrant cat. Pork samples are not to be relied on unless a considerable number, from different sources, are available.

The presence of the parasite is best determined by the microscopic examination of thin fragments of muscle from the diaphragm or from the base of the tongue, cut parallel with the fibers and examined under pressure. In the absence of a regular "trichina compressor" a very satisfactory substitute is furnished by two slides clipped together by spring clothes-pins. When an infection is located, bits of the muscle should be fed to white rats or mice, and one of these should be killed within 2 or 3 days for the study of the mature sexes isolated by the method described on page 108. A second should be examined 2 or 3 weeks later for wandering larvæ, and a third after 3 weeks for encapsuled larvæ. Calcified cysts will be present in muscle which has been infected for 6 months or longer.

Permanent mounts of these various stages should be made. Adult worms should be killed in hot 70 per cent alcohol, and gradually dehydrated and, after clearing in xylol, brought into thin balsam; or they may be mounted in glycerine gelatine and sealed, as described on page 105. Muscle containing the migratory stages may be macerated for 24 to 48 hours in 0.1 per cent chromic acid or 2 per cent acetic acid, teased, dehydrated, and mounted in balsam. For the cyst stages compressed muscle may be dehydrated and mounted in balsam; or it may be fixed, imbedded in paraffin, and sectioned. For ordinary study it is undesirable to stain the material.

To show the female worms *in situ*, the small intestine of a heavily infected rat should be removed a week or more after the experimental feeding, fixed in Bouin's fluid, sectioned, stained, and mounted by the usual methods.

While free larval stages are readily obtained by teasing infected muscle, an instructive demonstration is that of the action of the digestive juices on the cysts. For this purpose pieces of infected muscle the size of a pea are kept for a day or so at 38 to 40°C. in an artificial gastric juice consisting of: scale pepsin (U.S.P.), 0.25 gram; sodium chloride, 0.2 gram; hydrochloric acid (sp. gr. 1.19), 1 cc.; water, 100. If an incubator is not available the experiment may be carried out in a warm room.

For maintaining a supply of trichinæ, rabbits forcibly fed are preferable to rats. They are more resistant to the infection and are often more readily kept than are rats.

CHARACTERISTICS OF THE TRICHUROIDEA

To the superfamily **Trichuroidea** belong the famous trichina worm and the whipworm of man, several important species affecting the respiratory passages of carnivores, and a number of less important forms.

The group is characterized by the fact that the anterior end of the long body is prolonged into a slender, more or less whiplike portion, while the posterior end is more or less swollen and contains the genital organs. The œsophagus is very long and traverses a chain of large, single cells; there is no œsophageal bulb, and the anus is terminal. The ovary is single and the vulva is at the origin of the swollen part of the body.

We shall use as a type the trichina worm, *Trichinella spiralis*.

PRACTICAL WORK

Encysted Larvæ.—You will be furnished with a portion of infected muscle or with prepared slides showing the larval or cystic stages of trichina. If the former, snip with the scissors very thin sections of the muscle, lengthwise of the fibers, and mount under pressure between two slides held together by rubber bands or by clips. Search for the lemon-shaped cysts showing the coiled larvæ. Can you find cysts containing more than a single larva? More than two? It should be recalled that the cyst is formed from connective tissue elements of the host and not by the worms. If you have had the fresh material, supplement your study by examination of prepared slides. Make drawings illustrating the cysts and larvæ and their relation to the muscle tissue.

Some months after the formation of cysts in a host, their calcification sets in. This is the stage in which they were early noted as gritty particles in the flesh of cadavers in the medical schools of London. When examined with reflected light rather than by transmitted light, they appear chalky white. Study the demonstration specimens if you do not have a slide showing this stage.

Adults.—When trichinous flesh is ingested by man or other appropriate host, the capsules are dissolved by the gastric juice and the larvæ are liberated. If you have infected meat for study, some of it should be subjected to the action of an artificial gastric juice and kept at body temperature for a day or so, in order to obtain the free larvæ. Other portions should be fed to white rats or mice for study of the mature stages and the migratory larvæ. If such trichinous flesh is not at hand and not procurable, use the prepared slides which will be furnished.

Within 48 hours after being ingested the larvæ have developed into mature sexual individuals. Examine fresh or mounted preparations and observe the division of the body into the slender, elongate anterior end and the posterior swollen portion. Note the large cells making up the anterior portion and the delicate œsophagus piercing them. The more conspicuous alimentary tract of the swollen section opens by a terminal anus.

In the female worms the eggs may be seen in progressive development as they are traced forward from the posterior region to the vulva in the anterior fourth of the worm, through which the fully formed larvæ escape.

The males are only about half the size of the mature females and are readily distinguished by the presence of two caudal, hemispherical copulatory lobes. Between them are four minute papillæ.

Make drawings illustrating the structure of the male and female worms.

Migrating Larvæ.—By the end of the first week, larvæ are escaping in large numbers and are being carried by the blood stream and, to some extent, actively migrating to the muscular tissue. Tease parallel with the fibers small fragments of muscle from an animal which has been infected 2 to 3 weeks previously. Thus the still unencapsuled larvæ can be obtained in numbers. By the end of the third week cysts are being formed and the flesh is infective for new hosts.

Trichuris trichiura.—Examine the demonstration specimens of the whipworm of man (*T. trichiura*) or of the related species from the dog. In these forms the anterior portion of the body is excessively long and slender, suggesting a whiplash. Study and make drawings of the peculiar lemon-shaped brown eggs with pluglike clear sections at each pole.

References

Good general accounts of the structure and life history of *Trichinella spiralis* and of trichinosis, the disease which it causes, are to be found in present-day textbooks of zoölogy and of parasitology. Of the enormous literature on the subject, the following modern researches are of special interest and availability to American students:

RANSOM, B. H., 1916. Effects of refrigeration upon larvæ of *Trichinella spiralis*. *Jour. Agr. Research*, **5** (18): 819–854.

RANSOM, B. H., and B. SCHWARTZ, 1919. Effects of heat on trichinæ. *Jour. Agr. Research*, **17** (5): 201–221.

RANSOM, B. H., B. SCHWARTZ, and H. B. RAFFENSPERGER, 1920. Effects of pork-curing processes on trichinæ. *U. S. Dept. Agr., Bull.* 880, 37 pp.

CHAPTER IX

THE DETERMINATION OF HELMINTH INFECTIONS THROUGH FECAL EXAMINATIONS

TECHNICAL SUGGESTIONS

As an introduction to the methods of determination of parasites in the living host, this practicum is confined to the microscopic examination of fecal samples for helminth infections. Most of the eggs to be considered have already been seen in the course of the practicums, but it is essential that the student learn to identify them and to distinguish them from the miscellaneous debris of fecal samples.

If time and the size of the class permit, the entire preparation and examination should be carried out by each student. For this purpose fresh feces of cats and dogs, preferably of young animals, may be used.

Ordinarily it is necessary to rely on formalin-preserved material, and such may often be from human sources. Samples from infected patients can be secured through the aid of physicians and hospital technicians, sedimented, and preserved in a liberal quantity of 10 per cent formalin. If there is considerable coarse debris, the sample should be passed through a fine-meshed sieve. (The tea-strainers available at the five-and-ten-cent stores make a convenient tool for this purpose.) After each using they should be flamed or thoroughly washed to avoid contamination of subsequent samples.

Eggs of a number of species of helminths affecting man, or of very closely related species, may be obtained from the parasites of various animals and should be stored in formalin as individual samples. In order to obtain quantities of eggs for class work the samples may be increased by adding the teased uteri of mature specimens. Unknowns may be prepared by combining several species after the study of the separate samples.

As illustrations of useful forms from animals may be cited the ascaris of the pig, ascarids, hookworms, and trichurids of cats and dogs; Tænia and Dipylidium eggs from cats and dogs; Hymenolepis eggs from rats and mice, or, for more general study, from chickens. The sheep liver fluke affords a typical fluke egg, but even the ever available frog flukes may be used. The oöcysts of rabbit coccidia should be studied as illustrations of forms that may be confused with helminth eggs.

One or more of the important schistosomes of man should be available. Formalin-preserved material or prepared slides of eggs of these are obtainable from dealers.

Permanent mounts of the various eggs used in this practicum may be prepared by Looss' method (p. 109) and sealed with Noyer's cement.

METHOD OF FECES EXAMINATION

The determination of parasitism in the living host can rarely be made on the basis of clinical symptoms. It requires usually the microscopic examination of the various excreta, of the blood, and even of bits of living tissue. A large number of endoparasites inhabit the alimentary canal or glands, such as the liver, of their host and discharge their eggs or larvæ or segments in the feces. To some extent in man and commonly in animals, eggs of lung parasites are likewise swallowed and pass through the intestine. Their presence in the living host is then revealed by the examination of the feces. This examination may be both macroscopic and microscopic. The parasites concerned may be protozoal, helminth, or arthropod. In this practicum we shall restrict our attention largely to the helminths, and particularly to the recognition of the eggs of these forms.

Macroscopic examination of normal stools is very superficial and to be trusted only when it is positive. More definite information can be obtained by giving the patient a vermifuge and examining all of the stools for a period of at least 48 hours. The fecal material is broken up in a large quantity of water in a flask and as rapidly as it settles the supernatent fluid is decanted; or the stool is washed repeatedly through a fine-meshed sieve. The sediment is poured into a tray and examined for segments of tapeworm, small nematodes, and flukes. A black photographic tray is useful for this examination.

Microscopic Examination.—The simplest method of microscopic examination is to remove several samples of the stool, about the size of a pinhead, emulsify on a slide in clean water, and cover with an 18-mm. square cover glass. If the stool is quite fluid, it may not need dilution. Care should be taken not to have the preparation too opaque, for eggs and cysts may be overlooked. On the other hand, if it is too thin, time is wasted in search. A good rule is to have it thin enough for ordinary print to be read through the preparation.

Simple Sedimentation.—The search for microscopic evidences of parasitism may be considerably lightened by thorough and repeated sedimentation of stool samples. Finally small quantities of the sediment are mounted for examination.

Centrifuging.—An improvement on the method of simple sedimentation is thoroughly to emulsify in water a sample the size of a walnut, strain out the coarse particles, and then centrifuge for the purpose of giving a rapid and certain concentration.

Treatment with Dense Liquids.—Advantage may be taken of the fact that parasite eggs are of specific gravity different from that of the other constituents of the material to be examined. In hookworm work a small quantity of feces is placed in a vial three-fourths full of saturated solution of salt (NaCl), thoroughly shaken, and allowed to stand for an hour; then a drop from the *surface* of the fluid is examined. If the concentration is correct all of the eggs rise to the surface and large numbers may be found in a single drop. The process is much facilitated by forcing the coarse float below the surface with a disk of No. 0 steel wool.

The so-called *Willis method* is now widely used in hookworm campaigns. A saturated solution of NaCl is prepared in boiling water and allowed to cool. One or two grams of the fecal sample are then thoroughly mixed with this concentrated salt solution and the container then filled to the brim with it. A clean glass slide is placed in contact with the film and allowed to stand for 15 minutes. It is then carefully removed, inverted, and examined under the low power of the microscope for adhering eggs.

The various methods of treatment with dense liquids will not reveal the presence of operculate eggs such as those of most flukes and of the fish tapeworm of man.

PRACTICAL WORK

Prepare an emulsified sample as above described or mount under a square cover glass a small drop of sedimented fecal sample preserved in 10 per cent formalin solution.

Beginning at one corner of the preparation pass systematically from end to end under the microscope until the whole field has been covered. Distinguish carefully between fragments of plant tissue, partially digested muscle and other food, plant spores (see Fig. 18), and the eggs, larvæ and cysts of parasites. Vary the lighting of your preparation by the use of the iris diaphragm and note the great role which this procedure plays in the detection and identification of significant objects.

Using the following key, as explained by the instructor, determine the eggs found. Do this for each of the samples supplied for laboratory work. Note carefully the relative size of the eggs and draw them to scale.

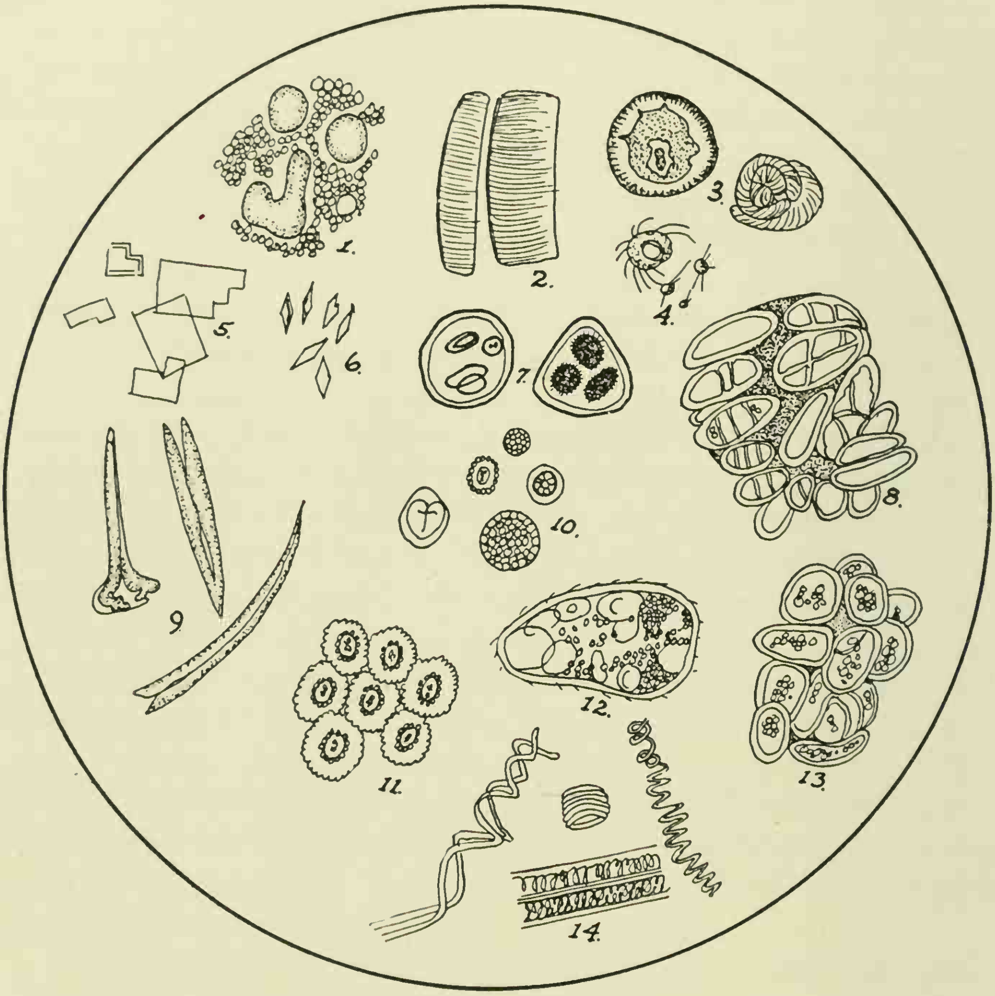

Fig. 18.—Microscopic appearance of common objects in the feces. 1, casein and fat droplets; 2, muscle fibers; 3, soap crystals; 4, crystalline fatty needles; 5, cholesterin crystals; 6, Charcot-Leyden crystals; 7, truffle spores; 8, portions of husks of cereals; 9, hairs of wheat grains; 10, spores of fungi; 11, cells from pericarp of peas; 12, parenchyma of beans; 13, endosperm of rice; 14, vegetable spirals. (*After Manson-Bahr.*)

Remember that an important part of this work is the recognition of plant cells, starch grains, and other food debris, pollen grains, spores of fungi, and the like, which are readily confused with eggs of parasites. Plant hairs and spiral plant tracheids should also be noted carefully as they are not infrequently mistaken for nematode worms.

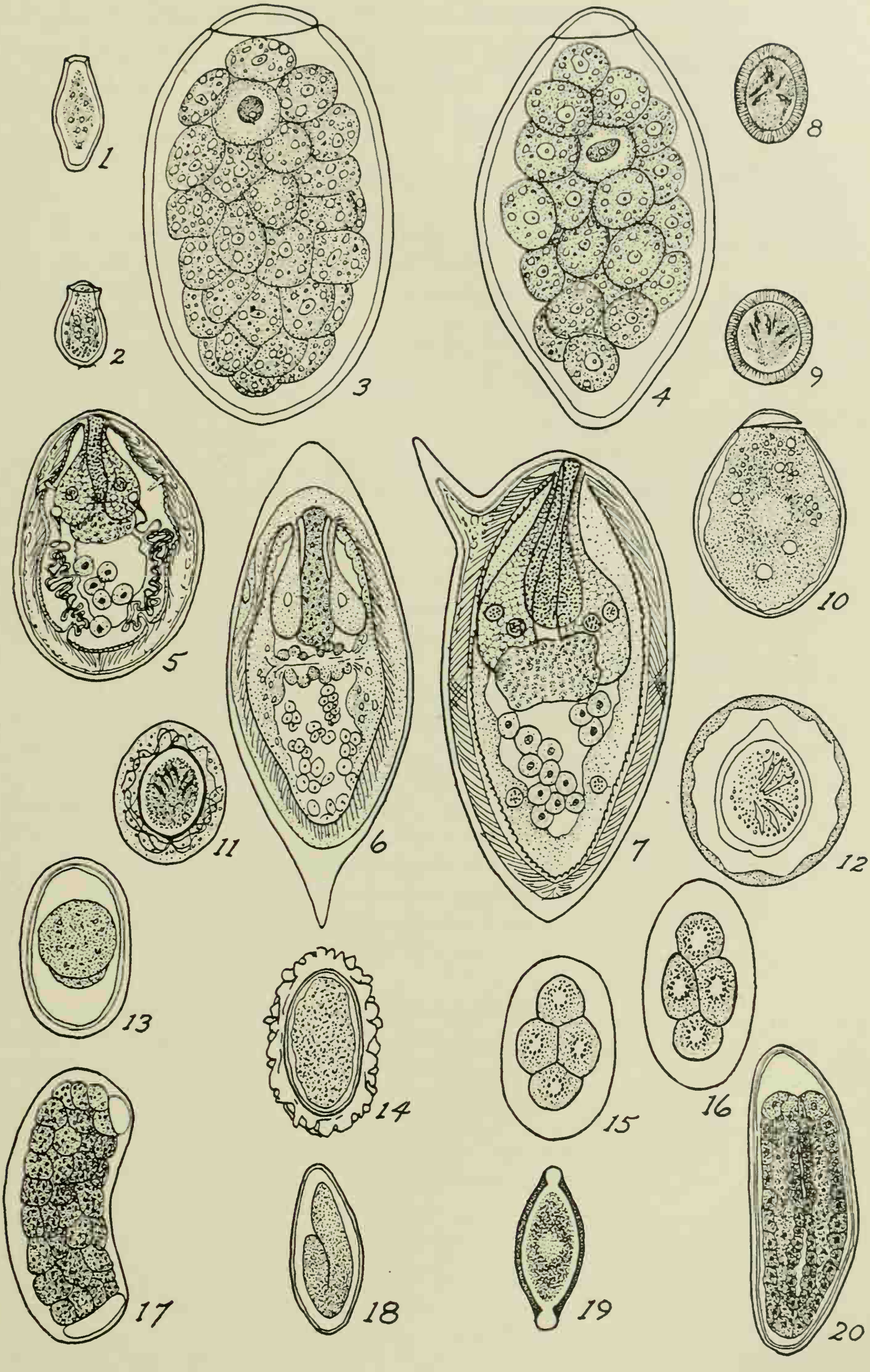
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FIG. 19.—Eggs of parasitic worms of man. 1, *Opisthorchis felineus;* 2, *Clonorchis sinensis;* 3, *Fasciola hepatica;* 4, *Fasciolopsis buski;* 5, *Schistosoma japonicum;*

KEY TO PARASITE EGGS IN HUMAN FECES

A. Eggs round or slightly oval, containing a six-hooked embryo.

B. Slightly oval with finely honeycombed surface; 65 to 75μ in diameter. Common in cat, very rare in man. *Toxocaramystax*

BB. Surface of egg not honeycombed.

C. Eggs isolated.

D. Brown or brownish in color with thick, radially striated capsule or embryophore; 30 to 40μ in diameter........................ *Tænia solium*
Tænia saginata

DD. Colorless with thin membranous inner shell, unstriated, separated from very thin outer membrane by a transparent, semifluid clear substance.

E. 30 to 60μ in diameter...... *Hymenolepis nana*

EE. 54 to 86μ in diameter...... *Hymenolepis diminuta*

CC. Eggs in rusty red packets of 12 or more. *Dipylidium caninum*

AA. Eggs not round, though they may be broadly oval.

B. Broadly oval, long axis less than twice that of shortest axis.

C. With mammellated, thick, glutinous capsule, size 50 to 80μ long, 45 to 55μ broad............... *Ascaris lumbricoides*

CC. Without such capsule.

D. With thick, smooth shell, size 75 to 85μ, contents undivided or in early segmentation stage. Common parasite of dog, rare in man...... *Toxascaris limbata*

DD. With delicate lid or operculum at one end.

E. Eggs large, exceeding 100μ in length, rounded at both ends.............. *Fasciolopsis buski*

EE. Eggs less than 100μ in length.

F. Eggs exceeding 80μ in length, broadly truncate at opercular end. Found in sputum as well as in feces. *Paragonimus westermani*

FF. Eggs less than 80μ in length, evenly rounded at ends, contents coarsely granular, mulberry-like, shell thin and light straw colored........... *Diphyllobothrium latum*

BB. Eggs not broadly oval, longest axis approximately twice the length of the shortest one.

C. Eggs more or less truncate.

6, *Schistosoma hæmatobium;* 7, *Schistosoma mansoni;* 8, *Tænia saginata;* 9, *Tænia solium;* 10, *Diphyllobothrium latum;* 11, *Hymenolepis nana;* 12, *Hymenolepis diminuta;* 13, *Ascaris lumbricoides*, without shell; 14, *Ascaris lumbricoides*, normal; 15, *Ancylostoma duodenale*; 16, *Necator americanus;* 17, *Heterodera radicicola* (Oxyuris incognita); 18, *Enterobius vermicularis;* 19, *Trichuris trichiura;* 20, *Syphacia obvelata.* (*Figures* 1, 3, 4, 6, 15, 16 *from Looss;* 8, 9, 13, 14, *Neumann and Mayer*; 5, 7, 18, *Cort;* 2, *Ward;* 12, *Grassi*, 10 *Brumpt;* 11, *Augustine;* 17, *Sandground;* 20, *Riley.*)

D. Slightly truncate at one end and the delicate operculum with shell projecting slightly behind its edge.

E. Egg relatively narrow, average size 30μ by 11μ.
Opisthorchis felineus

EE. Egg broader, 26μ to 30μ by 13μ to 16μ.
Clonorchis sinensis

DD. Truncate at both ends with dark brown shell, 50μ long, slightly pointed and tipped with a little shiny clear plug, content unsegmented....... *Trichuris trichiura*

CC. Not truncate.

D. Large eggs, 120 to 160μ long, bearing a sharp spine.

E. Spine terminal, eggs found in urine and more rarely in feces......... *Schistosoma hematobium*

EE. Spine lateral, eggs found in feces.
Schistosoma mansoni

DD. Eggs not spined.

E. Containing a miracidium, or ciliated embryo; size averaging 83μ by 62μ, sometimes with slight knob-like lateral thickening.
Schistosoma japonicum

EE. Miracidium not present; embryo tadpole-like or vermiform, or egg content in early segmentation stages.

F. Very delicate, transparent, asymmetrical shell with double contour, 50μ by 80μ containing well developed embryo.
Enterobius vermicularis (*Oxyuris*)

FF. Delicate, single-contoured shell, symmetrical, and with broadly rounded ends. A broad clear zone between the shell and the content; normally showing early segmentation stages, but never larvæ in fresh stools.

G. Ends somewhat pointed, size 75 to 90μ by 39 to 47μ.
Trichostrongylus orientalis

GG. Ends more broadly rounded, size 58 to 80μ by 35 to 52μ.
Necator americanus

Size 56 to 61μ by 34 to 38μ.
Ancylostoma duodenale

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CHAPTER X

THE EXAMINATION OF SMALL MAMMALS FOR ANIMAL PARASITES

TECHNICAL SUGGESTIONS

Few students nowadays have any experience in the dissection of mammals in their course in general zoölogy. Even those who do dissect a type form, such as the cat, rabbit, or rat, have little idea as to its parasitic fauna. Not only for these reasons but also because it adds zest and value to the work, it is desirable that the members of the class get supervised experience in the examination of animals for parasites. This work may well follow the detailed study of the various helminths.

While the cat has been selected as probably the most suitable form for this work, the directions are of general applicability. If opportunity affords, various small mammals, such as the dog, rat, and rabbit, may be used at this time. They should be killed before the laboratory period.

Stretching boards, thumb tacks, sponges, and one or more bone forceps should be at hand. If the students do not have their own dissecting sets, there should be provided at least a sharp scalpel, scissors, dissecting needles, and forceps for each group.

Facilities for the preservation of material should be provided and the details of the work assigned to the two or four students who will be working on each specimen. Emphasis should be placed on the importance of proper labeling and of keeping accurate data as to the source and number of the parasites and possible evidence of pathological conditions.

THE INCIDENCE OF PARASITIC INFECTIONS

The current opinion, even among zoölogists, as to the rarity of many parasitic forms is largely due to lack of a systematic examination of animal hosts. Particularly has this been true in this country, although there is a growing realization of the value of such studies from the viewpoint of both pure and applied science. Even now there are few sections of this country for which we have accurate data as to the incidence and variety of parasitism of even such ubiquitous animals as the dog and cat, this in spite of the fact that both of these animals play roles in the maintainence of some of the most important parasites of man and domestic animals.

We have seen the extent and variety of parasitic infection of the common frog. Of the mammals, the domestic cat serves as a convenient subject for similar examination. Over a hundred species of parasities have been listed for this animal, though of course in a given locality an individual animal might harbor very few representatives. The present practicum will illustrate a simple routine for the examination of any small mammal.

PRACTICAL WORK

Preliminary Examination.—Search the fur carefully for ectoparasites such as ticks, lice, and fleas. Place any of these in the bottles of 70 per cent alcohol provided, and record their presence. It should be recalled that lice and fleas serve as intermediate hosts of the double-pored tapeworm of cats, and hence if your animal harbors this worm a percentage of the insects would probably be infected.

Examine for evidence of mange, such as falling hair and wrinkled, crusty skin particularly about the ears and upper part of the neck. If these conditions are encountered, make scrapings of the crusts and search with the microscope for the causative mite, *Notoëdres cati.* Examine scrapings from the auditory canal for the ear mite, *Otodectes cynotis.* Mounting the scrapings in 5 per cent caustic potash will render the parasites more distinct.

Preparation for Dissection.—Stretch the animal on its back on a broad dissecting board or tray and tie its extended legs. Wet the fur of the abdomen and part it along the midline. Then insert a scalpel just under the skin in the midline of the throat and slit the skin to the anus. Loosen the skin and pin it down on either side. Being careful to avoid piercing the intestines, make a longitudinal cut through the abdominal muscles from the sternum to the pubis and a lateral cut along the last ribs, and pin out these flaps with the skin. With bone cutters (*not* with scissors) cut through the ribs of both sides and, completing the cuts with a scalpel, lift off the sternum and attached stumps of ribs.

Before proceeding further, examine the abdominal and pleural cavities for larval tapeworms or other parasites. An elongated plerocercoid 2 to 3 cm. long by 2 to 3 mm. broad has been described for the serous cavities of the cat under the generic name *Dithyridium.*

Examination of Special Organs.—In the following outline there will be mentioned only the more common or important species of parasites of the cat. Additional species and references to descriptions are listed in the Appendix, page 114.

Lungs and Bronchi.—Remove the lungs to a dish of water and examine for any abnormal conditions. Scrapings of the mucosa and bronchi should be examined microscopically for eggs of the fluke *Paragonimus* and the nematode lungworm *Capillaria ærophila.* The former are typical operculate eggs of flukes; the latter are very similar to the brown oval eggs with a clear stopper-like plug at each end which you have seen in the whipworm, *Trichuris trichiura.* Rarely, minute larval nematodes are to be found in these scrapings. They are immature forms of another nematode, *Ælurostrongylus abstrusus,* whose eggs are laid in the alveoli of the lung. The adults which are to be found in the smallest bronchi, measure 5 to 10 mm. Cut the lungs into fragments and search for adult worms of any of these species.

Heart.—The senior author has recorded one case of the presence of the filarial worm *Dirofilaria immitis* in the ventricles of the heart of a cat. It is quite possible that such cases are to be found in the South.

Stomach.—Slit open and spread out the stomach on a dissecting board and examine with the naked eye for any macroscopic forms. Not infrequently the cat ascarid *Toxocara mystax* will be found in this organ. Examine microscopically scrapings of the stomach mucosa for the interesting nematode *Ollulanus tricuspis,* a minute form measuring 1 mm. or less in length.

Intestine.—Cut the intestine into sections, slit them lengthwise, and pin down. In slitting, care must be taken to avoid injury to worms in the lumen. Collect and preserve the grosser forms such as ascarids and tapeworms. Then examine closely for threadlike hookworms and minute flukes. If tapeworms are present, note the isolated segments, particularly those of Dipylidium which are large, brick-red, and cucumber-seed shaped in the ripe condition.

Examine under the microscope samples of the content of the small intestine for eggs or early stages of helminths.

Gall Bladder and Liver.—Remove the gall-bladder to a dish, open, and mount some of the content, diluting with water.

Search microscopically for eggs of trematodes. Cut the liver in slices about a centimeter in thickness and press lightly so as to force any parasites out of the smaller branches of the gall ducts. The most probable find is the fluke *Amphimerus pseudofelineus*, an elongate, tapering form, measuring up to 20 mm. Remove any found and then place the slices in a large dish of water or physiological salt solution and examine from time to time for any parasites that have emerged. The process is hastened if the fluid is at blood heat.

Kidneys and Bladder.—The kidneys should be included in the examination, for important parasites occur in these organs in some species of animals. We are not aware that any have been reported for cats in this country. A species of *Capillaria*, long and slender like that from the lungs, is reported as occurring in the bladder.

Muscle.—Minute samples of muscle, especially from near the tendon of the diaphragm, should be compressed between two slides and examined for the cysts of *Trichinella spiralis*. The food habits of cats are such that they have frequent opportunities to become infected by this dangerous parasite and thus to play a rôle in its maintenance.

Summary of Results.—Toward the end of the period the results of the various examinations will be collected and summarized. Such records carefully obtained and checked will ultimately afford important data on the distribution and incidence of parasites of economic importance.

CHAPTER XI

THE AMŒBÆ OF MAN

TECHNICAL SUGGESTIONS

On account of the difficulty of obtaining living material illustrating the endomœbæ of man, and the necessity of using oil immersion lenses for the study of details, a survey course such as this must rely chiefly on demonstrations. Fortunately, well-prepared slides stained in iron hæmatoxylin are now available through several of the biological supply houses listed in the Appendix.

Through such sources, if not through some hospital clinic, it is usually possible to obtain fecal material in formalin, containing trophozoites and cysts of *Endamœba histolytica* and *Endamœbæ coli.* By adding a drop of iodine solution to the slide, much may be gained from the study of this material under the high power dry lens.

For study of *Endamœba histolytica* in the tissues, the most favorable materials are sections through the rectum of experimentally infected kittens. Such preparations are offered by several of the supply houses listed. For aid in the laboratory examination a blackboard sketch or chart showing the structure of the normal organ should be before the student.

In most sections of the country rats and mice and, to a less extent, frogs, are infected with intestinal amœbæ very similar to *E. histolytica.* Our large native cockroach, *Periplaneta americana,* frequently harbors *Endamœba blattæ,* the type of the genus. These species can be used to advantage for the study of motile stages. While it is assumed that the student has made a careful study of living specimens of non-parasitic amœbæ in his course in general zoölogy, it is nevertheless well to review this material.

CHARACTERISTICS OF THE RHIZOPODA

Within recent years it has become known that some of the most devastating diseases of man and animals are due to Protozoa. All of the classes of the phylum contribute important illustrations of parasitic forms. They inhabit organs, tissues, and cells, and even the nuclei of cells of their various hosts.

The phylum is usually divided on the basis of the development of locomotary organelles into four classes: the Rhizopoda, the Mastigophora, the Infusoria, and the Sporozoa. Many students of animal parasitology include as a fifth class the Spirochæta, an important group of exceedingly tenuous, spiral organisms regarded by some authorities as bacteria.

As typical of the *Rhizopoda*, or "root-footed" organisms, you have already studied the free-living *Amœba proteus.* It should be recalled that this organism is an irregular mass of protoplasm, constantly changing its shape during life by the pushing out of lobe-like *pseudopodia* which constitute the locomotary organelles. The body consists of a clear outer layer, the *ectosarc,* and a granular inner layer, the *endosarc.* Within the endosarc lie the *nucleus,* a *contractile vacuole,* and *food vacuoles* of varying size.

Of the numerous species of amœbæ which have been described as infesting man, three are of special significance: *Endamœba histolytica* as the cause of bloody dysentery, and *Endamœba coli* and *Endolimax nana* as common species which are often mistaken for the pathogenic form.

PRACTICAL WORK

Endamœba histolytica.—*Amœboid Forms.*—Examine under high power prepared stained slides showing the amœboid forms, or trophozoites, of this amœba. Note the size as estimated by that of red blood cells under the same magnification. If a micrometer eye piece is available, make more exact measurements. The specimens are usually rounded in stained material and show only slight differentiation into ectoplasm and endoplasm. The endoplasm is free from ingested bacteria but in acute cases may contain red blood corpuscles. As compared with that of *E. coli,* the cytoplasm is relatively homogeneous and without prominent vacuoles. In normal, well-stained specimens it appears as a ring-like structure approximately the size of a red blood corpuscle (7.5μ) or somewhat smaller, representing the very delicate nuclear membrane covered on the inner surface by a layer of very minute chromatin granules. At the center is a minute deeply staining granule known as the *karyosome.*

Amœboid Forms in Host Tissues.—A section of the large intestine showing amœbic infection will be furnished. Under low power locate the following layers of the intestinal wall: (1) an inner glandular layer with closely crowded, deeply staining nuclei, which is about one-fourth of the entire thickness of the wall; (2) a narrow band of muscles constituting the *muscularis mucosæ;* (3) a light-staining middle portion, the *submucosa;* and

(4) a thick outer, deeply staining layer of muscles. Examining the base of the glandular portion under high power note the numerous rounded, vacuolated amœbæ. The structure of their nuclei is not typical as they are rarely fixed in a favorable manner.

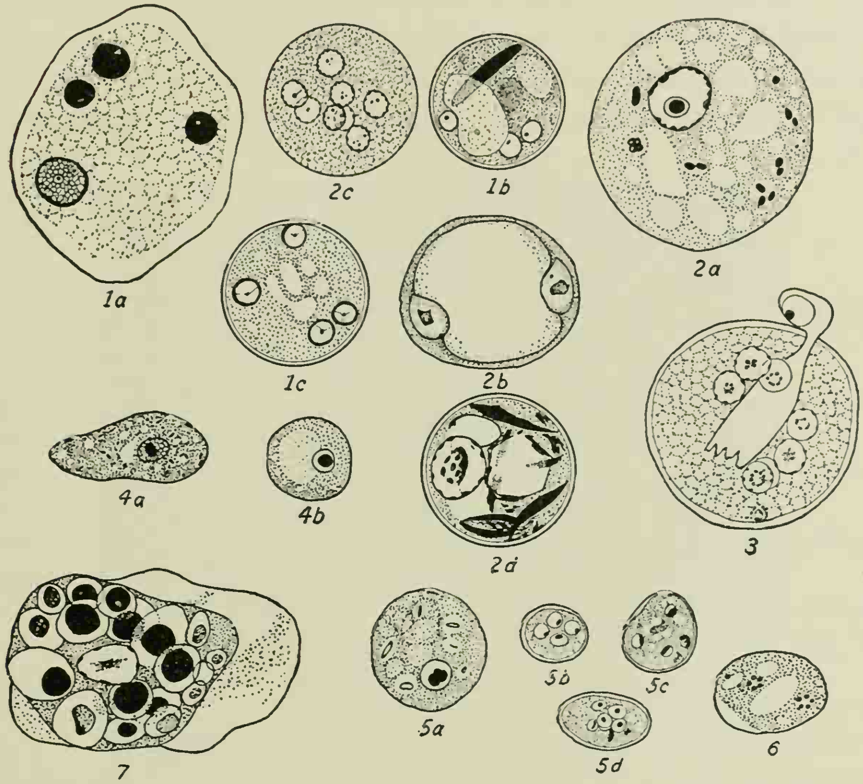

Fig. 20.—Amoebæ of man. 1*a*–1*c*, *Endamœba histolytica;* 2*a*–2*d*, *Endamœba coli;* 3, *Councilmania lafeuri* cyst with "bud;" 4*a*–4*b*, *Iodamœba williamsi;* 5*a*–5*d*, *Endolimax nana;* 6, *Dientamœba fragilis;* 7, *Endamœba gingivalis.* (*After Kofoid, except* 1*a and* 5 *after Boeck,* 2*b,* 2*c and* 4 *after Wenyon.*)

Cysts.—Search the prepared slides under high power for the round, four-nucleate mature cysts of *Endamœba histolytica.* They are sharply contoured, spherical bodies measuring from 5 to 20μ in diameter, whose nuclei show the typical delicate peripheral layer of chromatin and the central karyosome. In addition, about half of the cysts show one or more conspicuous black, rod-like bodies with rounded ends, which, like the chromatin of nuclei, stain black with the iron-hæmatoxylin stain. On account of this reaction they are called *chromatoid bodies.* In the same preparation will be found uninucleate and binucleate cysts. These contain one or more large glycogen masses which in fresh cysts stain a deep mahogany color in iodine. Make careful drawings of the various stages of cysts found.

For the examination of unstained cysts, formalin-preserved fecal material showing those of *Endamœba coli* will be used, since this species is more readily available than is *E. histolytica*. Mount a small drop of the sedimented material under a cover glass. Care should be taken not to use so much as to render the mount opaque. (A satisfactory preparation should be so transparent that ordinary print can be read through it.) Search for round, sharply contoured clear bodies, usually about twice the diameter of a red blood corpuscle. Add a drop of iodine solution and study under high power. The nuclei are brought out by the iodine and it will be seen that they are eight in number in the mature cysts as contrasted with four for those of *E. histolytica*. The karyosome is large and excentric and the peripheral chromatin, along the nuclear membrane, is thicker.

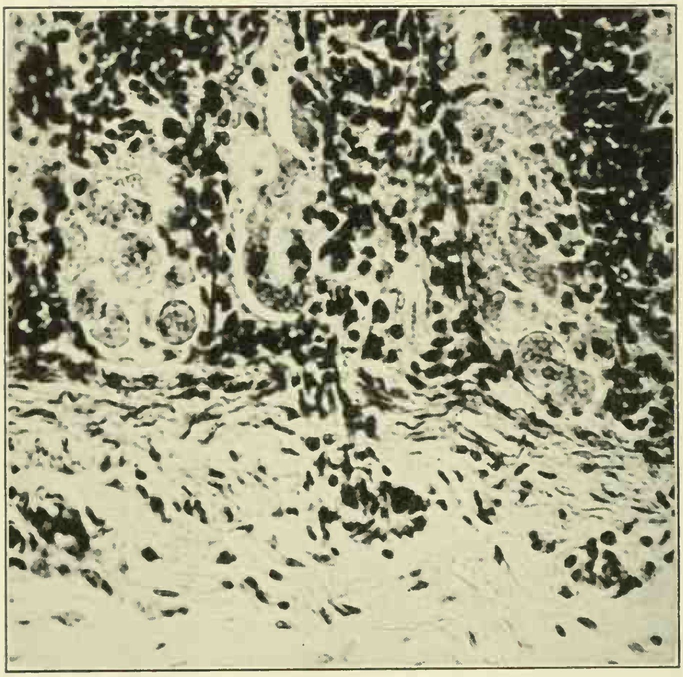

FIG. 20*a*.—Section of rectum of kitten infected by *Endamœba histolytica*, showing groups of the rounded parasites at the base of the crypts. (*Orig.*)

Preparations stained in iron hæmatoxylin frequently show splinter-like chromatoid bodies, in contrast to the rounded ones of *E. histolytica.*

Endamœba coli. *Amœboid Forms.*—Study the prepared slide of *Endamœba coli* and note that the peripheral chromatin of the nucleus is in a thicker layer and its granules coarser than in *E. histolytica.* The karyosome is coarser and excentric in position.

Of special significance is the fact that the endoplasm is much more granular and contains ingested bacteria, spores, and cellular debris.

Demonstrations.—Study the demonstration specimens of the trophozoites and cysts of the two species described above. Contrast with the trophozoites those of the common intestinal *Endolimax nana.* In this species the nuclear membrane is very delicate and the chromatin material is concentrated very largely in a central mass. The organism is small, the diameter averaging that of a red corpuscle.

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CHAPTER XII

TRYPANOSOMES AND ALLIED FORMS

TECHNICAL SUGGESTIONS

The most readily available trypanosome for laboratory work is *Trypanosoma lewisi* from the wild rat. A drop of fresh blood from the tail should be examined under the 4-mm. lens of the microscope, with the diaphragm almost closed. If a considerable number of freshly killed rats is available, transfer 5 to 10 cc. of blood to small vials, defibrinate, and examine promptly on return to the laboratory. In such blood, or in the livers of infected rats kept in a refrigerator, the trypanosomes will persist and be infective for a week or more.

If an infection is found, inoculate 3 to 5 cc. of the blood into the body cavity of a young white rat, by means of an ordinary hypodermic syringe or even by means of a fine-pointed pipette. The parasites appear in the circulating blood in a day or two and persist for upwards of a month. In order to be sure of maintaining the supply, other young rats should be inoculated at least once a month.

Detailed technique for the study of the life cycle of *Trypanosoma lewisi* in the rat flea may be found in the monograph by Minchin and Thompson, 1915.

Permanent mounts of trypanosomes may be prepared by the dry-film method, staining with Wright's stain. A preferable method is to fix the moist films in hot Schaudinn's fluid and stain in iron hæmatoxylin.

Prepared slides of *Trypanosoma gambiense* are on sale but offer no advantage over those of *T. lewisi* for general laboratory study.

Our native frogs are not uncommonly infected by *T. rotatorium*, but the parasites are rarely abundant. Hegner has called attention to the value of aquatic specimens of the salamander, *Diemyctylus viridescens*, as a source of *T. diemyctyli*.

Demonstration slides showing the intracellular phases of *Trypanosoma cruzi* are to be purchased, as are also those of *Leishmania*. As a representative of species having only an invertebrate host, *Herpetomonas muscæ-domesticæ* is readily obtainable from the intestine of houseflies and various blowflies.

CHARACTERISTICS OF THE MASTIGOPHORA

The **Mastigophora** or **Flagellata** are Protozoa which are provided with one or several whiplike flagella. The group is a large one and contains many free-living forms as well as numerous species parasitic in animals and plants. Of those infesting animals an important group of blood-inhabiting species and closely related forms belongs to the family **Trypanosomidæ.**

The **Trypanosomidæ** are characterized by a more or less spindle-shaped body, a central nucleus, and by the kinetoplast, from which arises the single flagellum. As a type we shall first study *Trypanosoma lewisi,* (Fig. 21) a cosmopolitan species found in the blood of the rat.

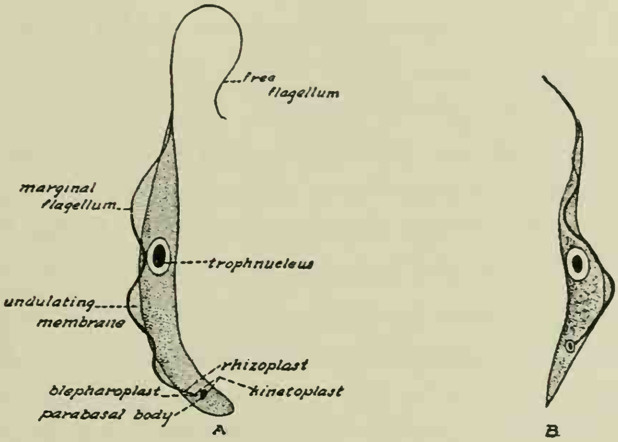

FIG. 21.—*A*, diagram of the structure of a trypanosome. (*Modified from Hartmann. B*, *Trypanosoma lewisi.* (*After Hartmann.*)

PRACTICAL WORK

Trypanosoma lewisi.—Examine under high power the prepared slide of rat's blood containing *Trypanosoma lewisi.* Do not restrict your study to a single specimen but choose the better examples. Note the general form of the body, and, using the red blood cells as an index, measure several specimens (the red cells of the rat average 6.2μ in diameter). The true nucleus is the larger, centrally placed, deeply stained body. Posterior to this, at the blunter end of the organism, is another nucleus-like body known collectively as the *kinetoplast.* Under high magnification it is seen to be made up of a larger, somewhat rod-shaped body known as the *parabasal body* and a minute, highly refractile granule, the *blepharoplast.* From this blepharoplast there arises the single flagellum, which is attached to the body by a characteristic *undulating membrane* as it runs forward to project as the free whiplike motile organelle.

Examine mounts of the fresh blood of an infected rat and study the movements of the numerous trypanosomes present.

Since trypanosomes occur in the blood of their host, the transmission to a new host involves a developmental cycle. In the case of *T. lewisi* it is undergone in the rat flea. It is not feasible to demonstrate this cycle in the present course, but it will be discussed in the lecture.

Demonstration Specimens.—Examine the demonstration slides of *Trypanosoma gambiense*, the organism of African sleeping sickness of man, and *Trypanosoma cruzi*, the cause of South American trypanosomiasis. In this latter species the parasites multiply not in the circulating blood but in the muscles and other tissue cells of the human host. Here they round up, lose their flagella, and by repeated division produce large numbers of forms resembling, in the possession of a nucleus and a parabasal body, the Leishmania organisms of kala-azar.

Leishmania.—Parasites belonging to this genus, often called the Leishman bodies, are minute Protozoa which are the cause of two types of disease in man: the one a generalized disease, kala-azar, and the other a cutaneous type known as oriental sore. The organisms as seen in the vertebrate host are very minute, oval bodies measuring 1 to 2μ in length and crowding endothelial or macrophage cells. In spite of their small size and their intracellular position they show their relationship to the trypanosomes by the presence of a distinct nucleus, a very distinct parabasal body, and a very slender rhizoplast which represents the basal portion of a flagellum. Study the demonstration slide of *Leishmania donovani*, the organism of kala-azar.

Trypanosomidæ from Invertebrates.—Since the blood-inhabiting trypanosomes of mammals typically undergo a cycle of development in an invertebrate host, different stages of the protozoan may be found on examination of these forms. In addition, however, insects and other invertebrates may harbor flagellates which, while showing relationship to trypanosomes, are apparently restricted to the one host. They are significant not only because of their resemblance to trypanosomes morphologically, and in their behavior in artificial cultures, but because of the probability that they represent the groups through which the blood-inhabiting species have developed.

These intestinal flagellates of invertebrates belong to the genera *Leptomonas*, *Herpetomonas*, and *Crithidia*. Like trypanosomes, they possess a more or less centrally placed nucleus and a single flagellum arising from a composite kinetoplast, which in these genera, lies anterior to the nucleus.

These will be illustrated by the widely distributed *Herpetomonas muscæ-domesticæ* from the intestine of house flies and related species. It is transmitted from fly to fly in the form of cysts in the feces. A much-studied *Leptomonas* is found in fleas, while the genus *Crithidia*, characterized by a rudimentary undulating membrane, is readily obtained from the intestine of the aquatic bugs commonly known as "water-striders."

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Excellent discussions of the trypanosomes and related flagellates are to be found in the texts of Craig; Hegner and Taliaferro; Hegner, Augustine, and Root; Thompson and Robertson; and especially of Wenyon. The following references indicate some of the sources of material for study.

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CHAPTER XIII

INTESTINAL FLAGELLATES OF VERTEBRATES

TECHNICAL SUGGESTIONS

Where hospital facilities permit the examination of considerable numbers of fresh stools, the species of intestinal flagellates infecting man may be used for this study. The more dependable sources are domesticated and laboratory animals.

Frogs commonly harbor in the rectum and at the junction of the large and small intestine *Eutrichomastix batrachorum*, *Trichomonas batrachorum*, and *Trichomonas augusta.* Kofoid and Swezy found the last-mentioned species abundantly in the salamander *Diemyctylus torosus* as well as in *Rana pipiens*, *R. boylei*, *R. draytoni*, and *Hyla regilla*, at Berkeley. They kept specimens alive for several months by diluting the intestinal smear with physiological salt solution and sealing the cover glass with vaseline. Cultures of various Trichomonads were made by placing a bit of the intestinal content in a hollow-ground culture slide, filling the cavity with physiological salt solution, and sealing the cover glass with vaseline.

Laboratory white mice and rats are frequently infected with *Trichomonas muris.* Less frequently it is found in house and field mice. *Trichomonas caviæ* is occasionally harbored by guinea-pigs.

An important source of material is the domestic chicken, whose cæca may yield the four species: *Chilomastix gallinarum*, *Trichomonas gallinarum*, *T. eberthi*, and *Eutrichomastix gallinarum.* Becker, 1926, reports on seven species of flagellates from the striped ground squirrel, *Citellus tridecemlineatus.*

Chilomastix species are reported from numerous other animals including rabbits and rats. They are commonly present in human dejections following the use of saline purgatives.

Giardia lamblia is a widely prevalent species affecting man. It is especially prevalent among young children, and the examination of an institutional group is a ready method of obtaining cysts. Motile and cystic stages of closely related species are very commonly found in rats, mice, guinea-pigs, and various other mammals. An interesting species is found in tadpoles.

In addition to the material from various animals used in the laboratory work, slides of *Trichomonas hominis*, *Chilomastix mesnili*, and *Giardia lamblia* should be available for demonstration. As opportunity presents, fecal material containing cysts of *Chilomastix* and *Giardia* should be preserved in 10 per cent formalin for diagnosis.

Unfortunately, the Giardia cysts break down in this fluid after a few months.

Permanent slides of any of the species to be used in this exercise should be made by fixing moist smears in hot Schaudinn's fluid and staining in iron hæmatoxylin (see p. 99). When possible, smears should be made from a fragment of the infected intestinal wall as well as from the intestinal content. Special care should be observed in differentiating the stain.

CHARACTERISTICS OF INTESTINAL MASTIGOPHORA

Of the many forms of intestinal flagellates we shall give special attention to three genera—Chilomastix, Trichomonas, and Giardia—not only as widely distributed forms, but also as genera represented by parasites of man. The consideration of them together is only a matter of convenience for, from a systematic viewpoint they represent distinct families. One of them, *Giardia,* is a member of a special order of Mastigophora, the **Diplomonadida,** or "double monads," characterized by a bilateral symmetry of the body, with two nuclei, eight flagella, and all of the organelles paired. The distinguishing characteristics of this and of the **Chilomastigidæ** and **Trichomonadidæ** will be brought out in the laboratory work.

PRACTICAL WORK

Examine, when possible, both living and stained specimens of the following representative parasites or related species from animals. Note the size, movements, structures, features, and the characteristics of the cysts when present.

Chilomastix mesnili is a fairly common parasite of the small intestine of man which has often been confused with *Trichomonas intestinalis.* It is pear-shaped with a rounded anterior end and a pointed posterior end. It has a characteristic jerky, spiral movement. The size varies greatly, the average being about 14μ in length by 6μ in breadth. The relatively large, round nucleus is at the very anterior end, dorsal to the cytostome ("mouth"). The three anteriorly projecting flagella should be examined in favorable specimens, with careful adjustment of the iris diaphragm of the microscope. In properly stained specimens under high magnification it may be seen that they arise from minute, dotlike blepheroplasts. A fourth flagellum lies within the large elongate cytostome and probably functions chiefly in obtaining food, as suggested by Boeck. The cytoplasm contains bacteria and other food inclusions.

The cysts are small, refringent, and pear shaped, with a transparent wall which is thicker at the anterior end. They measure from 7 to 9μ in length by 4.5 to 6μ in width. In the granular cytoplasm may be seen the large rounded or oval nucleus and the outlines of the cytostome with its fibrillar apparatus.

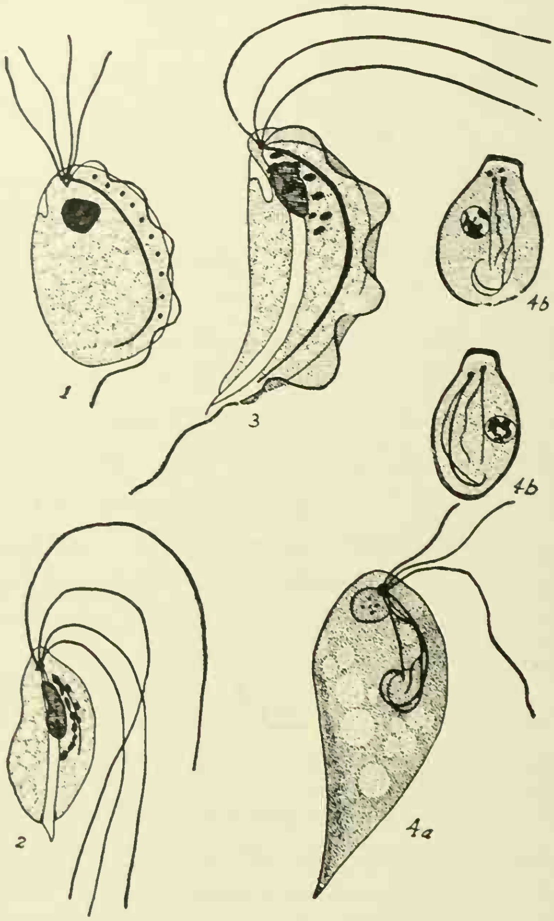

FIG. 22.—Intestinal flagellates of the chicken. 1, *Eutrichomastix gallinarum;* 2, *Trichomonas gallinarum;* 3, *Trichomonas eberthi;* 4, *Chilomastix gallinarum,* motile and cyst stages. (*Figures* 1–3 *from Martin and Robertson. Figs.* 4a *and* 4b *from Boeck and Tanabe.*)

Trichomonas hominis is said to be the most common flagellate affecting man. Statistics are not altogether reliable on account of the above-mentioned confusion in many cases with *Chilomastix mesnili.* More available species for study are *Trichomonas augusta* and *Trichomonas batrachorum* from the frog. Mount in a drop of water the intestinal content from the juncture of the small and the large intestine. The trichomonads may be seen as actively moving, tadpole-shaped organisms. Under high power, study a less active specimen. Cut down the light and note the three anteriorly projecting flagella and the posteriorly directed flagellum attached to the body of the organism by an undulating membrane. A wave of movement from the anterior to the posterior end of the flagellum and membrane simulates a series of minute projecting legs. At the anterior end, near the base of the flagella, is a small cytostome; while through the center of the body there runs a hyaline rodlike structure, the axostyle.

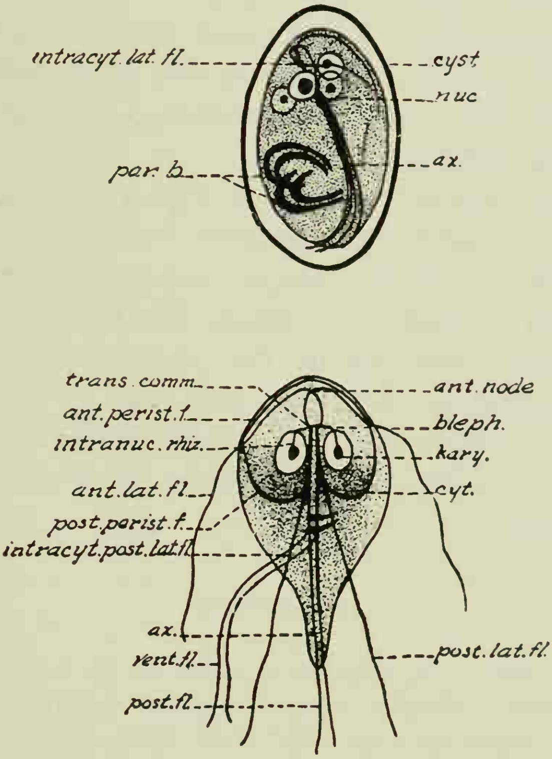
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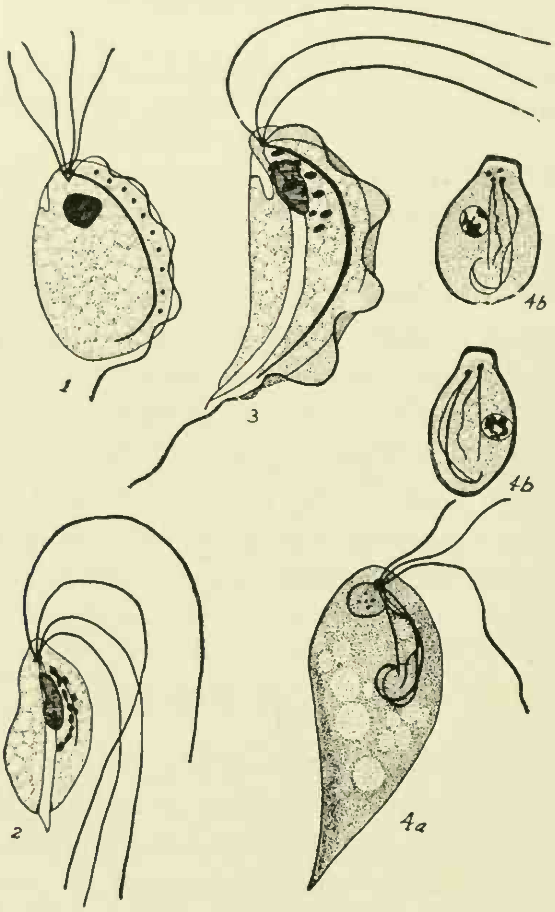
Fig. 23.—Ventral view and cyst of *Giardia lamblia*. (*After Kofoid and Swezy.*)

In stained specimens note the above-described structures and, particularly, the blepheroplast from which the flagella originate, the chromatic basal rod, and the nucleus. (*Cf.* Fig. 22.) The oval cysts with remains of the fibrillar apparatus will be demonstrated. Those of the species infecting man have not been found and it is generally believed that this species is transferred in the trophozoite stage.

Giardia lamblia (Fig. 23) is a remarkable parasite infecting man. In the older literature it is known as *Lamblia intestinalis* and hence the term *lambliasis*, as well as *giardiasis*, is applied to the infection. Occasionally it is transferred from man to chiefly in obtaining food, as suggested by Boeck. The cytoplasm contains bacteria and other food inclusions.

The cysts are small, refringent, and pear shaped, with a transparent wall which is thicker at the anterior end. They measure from 7 to 9μ in length by 4.5 to 6μ in width. In the granular cytoplasm may be seen the large rounded or oval nucleus and the outlines of the cytostome with its fibrillar apparatus.



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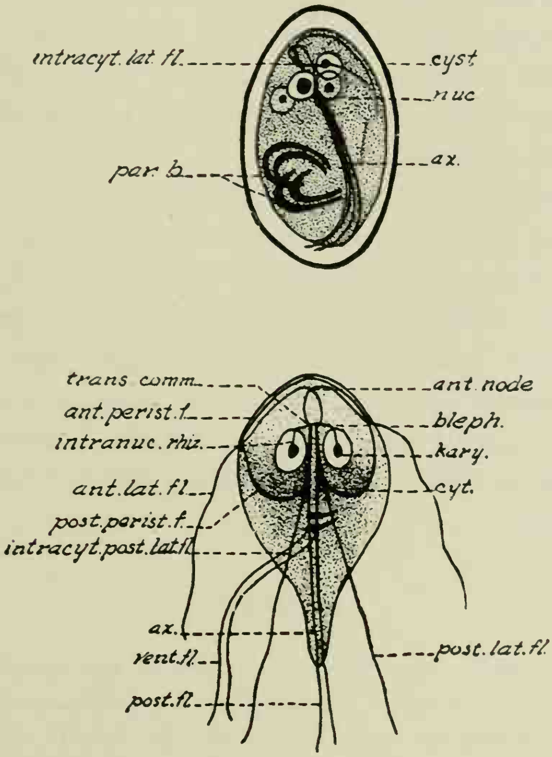
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FIG. 23.—Ventral view and cyst of *Giardia lamblia*. (*After Kofoid and Swezy.*)

In stained specimens note the above-described structures and, particularly, the blepheroplast from which the flagella originate, the chromatic basal rod, and the nucleus. (*Cf.* Fig. 22.) The oval cysts with remains of the fibrillar apparatus will be demonstrated. Those of the species infecting man have not been found and it is generally believed that this species is transferred in the trophozoite stage.

Giardia lamblia (Fig. 23) is a remarkable parasite infecting man. In the older literature it is known as *Lamblia intestinalis* and hence the term *lambliasis*, as well as *giardiasis*, is applied to the infection. Occasionally it is transferred from man to rats, but the very closely related species commonly infesting rats is *Giardia muris*. Other related species are found in rabbits, guinea-pigs, and numerous other animals. The organisms are the shape of a longitudinally cut half of a pear with a large concavity, or sucking disk, at the anterior end. They move in a characteristic manner, rotating on their long axis in a jerky manner. The size and relative breadth vary with the species, though considerable variations occur in the same species. A striking peculiarity of representatives of the genus is the duplication of organelles. There are four pairs of posteriorly directed flagella, two prominent nuclei, and two rodlike longitudinal axostyles. Just posterior to the sucker is an obliquely placed, deeply staining mass which represents a pair of more or less fused parabasal bodies.

The cysts are hyaline, oval bodies with a well-marked refractile membrane and exhibit two or more nuclei and traces of the fibrillar apparatus in the living condition. Specimens well stained in iron hæmatoxylin show the basal portions of the flagella and the axostyles in the younger cysts. There are usually four nuclei in the older ones, and in some cases eight and even more have been noted. Near the posterior part of the cyst are paired, coarser, deeply staining fibrils whose significance is still in dispute.

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CHAPTER XIV

A SIMPLE LIFE CYCLE OF A SPOROZOAN

TECHNICAL SUGGESTIONS

The species of *Monocystis* from the seminal vesicles of the earthworm, on account of their availability and the readiness with which the various stages can be found, afford a most satisfactory introduction to the study of the Sporozoa.

In most localities the great majority of the large *Lumbricus terrestris* will be found infected. In regions where this worm is not established, it is desirable to purchase living specimens from some Eastern collector or through supply houses. In case this species is not obtained, local species will usually be found to harbor related parasites, as shown by Mickel, 1925, who found *Helodrilus caliginosus* in Minnesota heavily infested by *Zygocystis cometa.*

For supplementing the study of fresh materials, sections and smear preparations should be prepared from heavily infected specimens.

Grasshoppers, cockroaches, and the much-used mealworms (larvæ of *Tenebrio molitor)* are certain sources of cephaline gregarines. The mealworms can be purchased from bird dealers if a supply is not kept in the laboratory.

CHARACTERISTICS OF THE GREGARINIDA

The class *Sporozoa* is a somewhat heterogeneous group of Protozoa, made up of forms exclusively parasitic, which lack definite locomotory organs, mouth, anus, and contractile vacuoles. They produce at some stage in their life history resistant spores which, however, are not directly infective but give rise to *sporozoites* which are the forms infecting new hosts.

The order **Gregarinida** includes cœlom-inhabiting Sporozoa which reproduce typically by spore formation after the fertilizing union of similar gametes. They are very common as parasites of invertebrates, chiefly insects, but the majority of the species are probably harmless to their hosts. Dissemination is usually passive, infection being by way of the alimentary canal.

In spite of their relative unimportance as parasites, the generalized life cycle of the Gregarinida and their close relationship to important groups of mammalian and human parasites make them important objects of study. Among the most widely distributed and best known forms are *Monocystis agilis* and related species from the seminal vesicles of earthworms. In many localities practically every worm is infested and all stages may be found.

PRACTICAL WORK

Directions for Dissection.—A fresh specimen of *Lumbricus terrestris* or related species of earthworm will be supplied. Place the worm in a flat-bottomed tray with the dorsal surface upward, stretching with a pin through the fifth segment and in the neighborhood of the thickened clitellum. Very carefully slit through the skin along the mid-dorsal line, avoiding piercing the alimentary canal or other organs. The conspicuous cream-colored bodies at the side of the œsophagus, and overlying it in the ninth, tenth, and eleventh segments are the seminal vesicles.

Remove with forceps a bit of one of the seminal vesicles and tap it in a drop of physiological salt solution (or water) on a slide. Cover the preparation and observe and make careful drawings of the following:

Normal Tissues.—Preparatory to study of the parasitic organisms, distinguish and make drawings of the following normal tissues of the seminal vesicle: (1) the sperm morulæ, spherical masses of small rounded cells which should be studied both in surface view and in optical section; (2) mature spermatozoa in brush-like clusters attached to a central core of protoplasm; (3) epithelial cells, flattened, angular, and homogeneous.

Monocystis sp.—Having distinguished the normal tissues in your preparation, search out various stages of the gregarine parasites. Study and figure the stages as you find them, but arrange your drawings in the following order:

1. *The young trophozoite,* or feeding stage, is a minute nucleated body in the midst of a sperm morula. As it develops at the expense of the sperm cells it becomes elongated and finally appears as a spindle-shaped organism with a cilia-like covering of short, abortive spermatozoa.

2. *Motile trophozoites,* extended and contracted, may be found in the seminal fluid. The living organism exhibits slow. flowing movements. Note the clear ectosarc, granular endosarc, and nucleus.

3. *Gametocytes.*—Two similar individuals become associated, and though remaining perfectly distinct from one another, secrete about themselves a common envelope, or *cyst.*

4. *Gametes.*—The nucleus of each gametocyte breaks up into a large number of nuclei. These become arranged around the periphery of the gametocytes, and about each nucleus there is cut off a little of the protoplasm of the mother cell. This nucleated mass of protoplasm is now known as a *gamete,* or sexual element. A large number of these gametes are formed, but a portion of the original cell remains as *residual protoplasm,* or "Restkörper." Study these points in prepared sections through infested seminal vesicles.

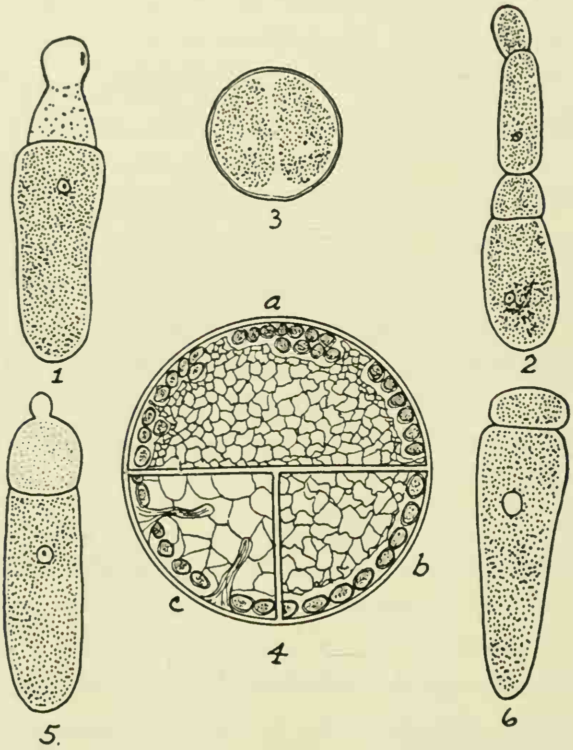

Fig. 24.—The gregarine parasites of the meal worm, Tenebrio molitor. 1–4, *Gregarina cuneata;* 2, conjugating individuals; 3, gametocytes encysted; 4, diagram of spore formation. In *a,* the gametes formed by the gametocytes are fusing to form sporoblasts, in *b* and *c,* the spores are maturing. 5, *Gregarina polymorpha;* 6, *Gregarina steini.* (*After Berndt.*)

5. *Sporoblasts.*—The gametes now pass from one cell to the other and conjugate in pairs to form rounded sporoblasts. The nuclei fuse and the sporoblasts become oval and form a spore.

6. The *spore* in Monocystis is boat shaped and is often known as a *pseudonavicula.* As in the Sporozoa in general, this is not the infective stage; but the nucleus divides and the protoplasm forms sporozoites.

7. The *sporozoites* vary in number, depending upon the genus. In Monocystis the longitudinal division of the spore content into sporozoites can be indistinctly seen in the living spore if the light is properly adjusted. After studying it in this manner, examine sections of the seminal vesicles showing spores in cross and longitudinal sections. Make drawings showing the sporozoites and the granular "residual protoplasm."

Other species of Monocystis may occur and should be figured if found. A related genus, *Zygocystis,* has the adult trophozoites more or less pearshaped, with frayed ends, and always united in twos and threes. Very many species of Gregarinida are known, of which the majority are parasitic in insects.

Nematodes.—In the course of these studies roundworms are occasionally to be found in the seminal vesicles of the earthworm. These are *Discelis filaria* of Dujardin, 1845, or closely related species.

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CHAPTER XV

THE LIFE CYCLE OF THE COCCIDIA

TECHNICAL SUGGESTIONS

The most readily available species for the study of the life cycle of a coccidian are *Eimeria stiedæ* and *E. perforans* from the rabbit, the former from the liver and the latter from the intestine. By some writers the two are considered identical and the following laboratory outline is applicable to either.

Infections may be detected by examining the feces of laboratory rabbits, even if acute cases are not available. The oöcysts may be concentrated by breaking up the feces in water, sedimenting repeatedly, and straining out the coarser particles. They are then cultured to the infective stage in Petri dishes under a thin layer of 4 per cent potassium bichromate solution to prevent mold. Andrews, 1926, comminuted heavily infected feces in 1 per cent chromic acid, cultured for a week or so, strained out the coarsest particles of feces through cheesecloth and then through batiste, and finally floated up the oöcysts by saturation with sodium chloride. If acute cases are available the animal may be killed and the oöcysts recovered by scraping the infected mucosa, thoroughly shaking the scrapings to remove the oöcysts, and then centrifuging gently for one or two minutes so that the oöcysts are thrown down while the cell debris remains suspended. Freshly recovered oöcysts as well as those which have undergone development to the infective stage should be preserved in 10 per cent formalin for class study.

Others should be administered, in food or by drenching, to young rabbits, in which acute cases of coccidiosis may be induced for further study. For critical work Andrews administered known numbers of the oöcysts by means of a stomach tube. Portions of the diseased liver or intestine, dependent upon the species of parasite, should be fixed in saturated corrosive sublimate solution and in picro-formol, sectioned, and stained in hæmotoxylin and eosin.

In lieu of rabbit material young chickens often afford an abundant supply of *Eimeria avium*. Myriapods of the genus *Lithobius* commonly harbor *Eimeria schubergi*, but it is generally associated with other forms which may confuse the study of it. English sparrows commonly yield a species of *Isospora* and others of this genus are readily obtainable from cats, dogs, foxes, and various other mammals.

CHARACTERISTICS OF THE COCCIDIA

The *Coccidia* are cell-infesting Sporozoa which typically reproduce intracellularly by asexual spore formation (*schizogony*) as well as by true *sporogony,* thus having a life cycle with alternation of asexual and sexual generations. The former provides for the multiplication of the parasite within the host, while it is through sporogony that infection of new hosts is brought about. After fertilization the *oösphere* forms sporoblasts which may or may not be covered by a sporocyst membrane, and which may each become transformed into one or several sporozoites.

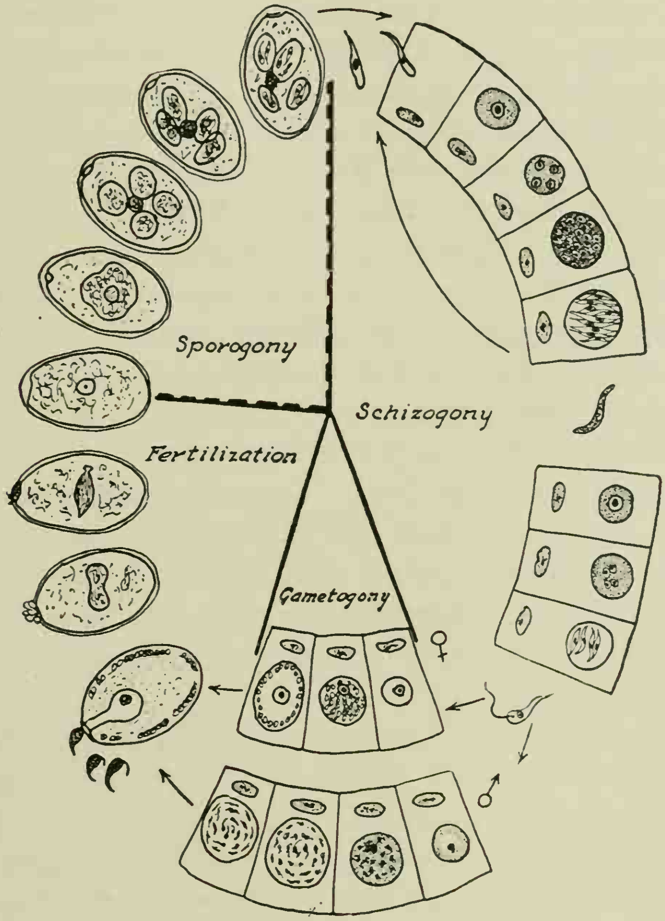

Fig. 25.—Diagram of the development cycle of a Coccidian. (*After Reich.*)

The chief difference between the Coccidia and the Gregarinida, as typified by Monocystis, is then, the intracellular habitat of the parasites and the interpolation of an asexual cycle.

There are many species of Coccidia infesting both invertebrates and vertebrates, including man. We shall use as a type *Eimeria stiedæ,* a species which commonly infests rabbits and is the cause of much mortality, particularly among the young animals. Related species cause serious disease of cattle, dogs, birds, and other animals. Several species are reported for man but infections are rare.

PRACTICAL WORK

Oöcysts.—Coccidial infections are conveyed from animal to animal by oöcysts which pass out with the dung. We shall begin our study with these bodies as they appear when first discharged. Note their oval form, the size, the sharply contoured envelope, and the minute pore, or *micropyle*, at one end. The protoplasmic content may fill the cyst in the early stages or may be later clumped into a spherical mass.

Development outside the Host.—The oöcysts are incapable of further development until they have been discharged from the body of their host. In the presence of oxygen and under favorable conditions of temperature and moisture the protoplasm divides into four *sporoblasts.* These secrete cyst walls and become *spores,* each of which produces two *sporozoites.* The infective oöcyst thus contains four spores and eight *sporozoites.* Make drawings illustrating the various stages.

Development within the Host.—When ripe oöcysts are ingested by a rabbit, the sporozoites are liberated by the action of the gastric and pancreatic juices and bore into the intestinal or gall-duct epithelium. Here they undergo asexual multiplication, or schizogony, and the early stages of their sexual reproduction, or sporogony.

In prepared slides of infected intestine study the following:

Schizogony.—When the sporozoites enter the epithelial cell, they become rounded *schizonts* and begin to increase in size. In unstained preparations they are readily distinguished from the host cell by their greater refractivity. As the schizont develops, the nucleus divides repeatedly and finally the schizont breaks up into a number of nucleated merozoites. These merozoites are actively motile and enter new epithelial cells, become schizonts, and continue the cycle. The infection may be so extreme that large areas of tissue of the host are destroyed.

Sporogony.—The infection of new hosts is made possible by the sexual cycle, or sporogony. Certain merozoites instead of developing into asexual schizonts develop as male or female gametocytes. Unlike those of the Gregarinida, there is a marked difference in the gametes and hence the cells producing the male elements are known as *microgametocytes*, those producing the female gametes are designated *macrogametocytes*.

Microgametocytes.—The microgametocytes present a clear, finely vacuolate protoplasm and reach a much greater size than either schizonts or macrogametocytes. From each there arises a great number of microgametes analogous to the spermatozoa of the higher animals. A voluminous mass of residual protoplasm remains unchanged.

Macrogametocytes.—The female gametocytes possess a coarsely granular, dense protoplasm, rich in nutrient substances. In sections the granules, arranged around the periphery, are so large as to resemble nuclei. The true nucleus is, however, clearly evident as a large central body. The young macrogametocytes are spherical, but they become oval as they mature. They undergo a process of maturation, after which they are known as macrogametes.

Oöcysts.—The mature macrogamete is fertilized by a microgamete and through the fusion of their nuclei, the oöcyst is formed, ready to continue its development when it passes from the host.

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CHAPTER XVI

THE HÆMOSPORIDIA

TECHNICAL SUGGESTIONS

For most classes it is necessary to precede the study of malarial parasites and related forms by a study of the morphology of the blood, with particular attention to the types of leucocytes. Otherwise, the student is very apt to confuse the nuclei of these cells with the parasites for which he is searching.

In this country it is rarely possible to demonstrate the living malarial parasites of man. If such an opportunity presents itself, a preparation should be sealed with vaseline and movements of the organism and pigment granules demonstrated. Mature parasites if kept on a warm stage may be seen to sporulate.

Stained preparations of *Plasmodium vivax* and *P. falciparum* are widely obtainable from dealers. Less frequently, demonstration specimens of *P. malariæ* may be purchased. In some parts of the country endemic malaria still occurs, and even in malaria-free sections the widespread use of induced malaria in treatment of paresis affords occasional opportunities for obtaining material from hospitals and clinics. In such cases smears should be made at 12-hour intervals beginning immediately after a chill and continuing through the cycle. Stain with Wright's stain (p. 110).

Plasmodium præcox and related species are found in English sparrows in this country, although natural infections do not seem common. Laboratory strains have long been maintained in canaries in various laboratories and infected birds may be obtained from some of the dealers listed on page 124. The parasites are abundant only during the acute stages of the infection and hence fresh birds should be inoculated a week or so before needed for laboratory work. This is done by pricking a vein under the wing or in the leg of the infected bird, sucking up a drop of blood into a syringe containing physiological salt solution, and injecting into the breast muscles of the clean bird.

The sexual cycle in the mosquito can be obtained by the methods given in detail by Huff, 1927. Reared females of *Culex pipiens* are kept away from water for 24 hours and then allowed to feed on heavily infected canaries at night. The feathers of the bird should be parted in the pectoral region and wetted down. It is immobilized by tying it snugly in a piece of netting and then placed on the crinoline-gauze top of a lantern-chimney breeding cage in such a way that its exposed breast is accessible to the mosquitoes. A strip of cloth is placed over it and extended down along the side of the lantern globe and held firmly in position by a rubber band. The engorged insects are then removed to another breeding cage and maintained on soaked raisins for varying periods before dissection. The oöcysts reach their maximum size about the tenth day.

Crows are very commonly infected by Hæmosporidia of the genus Hæmoproteus (Halteridium), the forms in which McCallum first worked out the significance of exflagellation. With living material from this source the formation of the microgametes and the process of fertilization can readily be demonstrated.

Demonstration slides of *Babesia bigemina* and *Babesia canis* can be purchased.

For the practical work outlined it is, of course, desirable to use oil immersion lenses. If this is not feasible it is quite possible to make the study with the aid of high power dry lenses, supplemented by demonstrations.

CHARACTERISTICS OF THE HÆMOSPORIDIA

The order **Hæmosporidia** includes the blood-dwelling Sporozoa, intracorpuscular or free in the plasma, and with or without alternation of hosts. From the zoölogical viewpoint as well as from that of human pathology the most important are the malarial parasites belonging to the genus *Plasmodium.* Related species of the same genus occur in sparrows and finches. Of much importance in our Southern states is *Babesia bigemina,* the organism of Texas fever of cattle.

PRACTICAL WORK

There will be furnished a slide of blood from a case of benign tertian malaria, *Plasmodium vivax.* The preparation has been stained in Wright's stain. Preparatory to the search for malarial parasites in the slides furnished it is necessary to become acquainted with the normal elements of the blood. Particularly is it desirable to study the blood platelets and to distinguish the types of leucocytes. For this purpose compare your findings with the chart (Fig. 26).

Plasmodium vivax.—Having completed this preliminary examination, begin at one corner of the preparation and search carefully the entire slide for various forms of the malarial parasite in the erythrocytes, or red blood corpuscles. If the case from which the slide was prepared was one of simple tertian malaria, the parasites will be in approximately the same stage and slides from other cases must supplement each other. Not infrequently double or even triple infections may occur from bites of infective

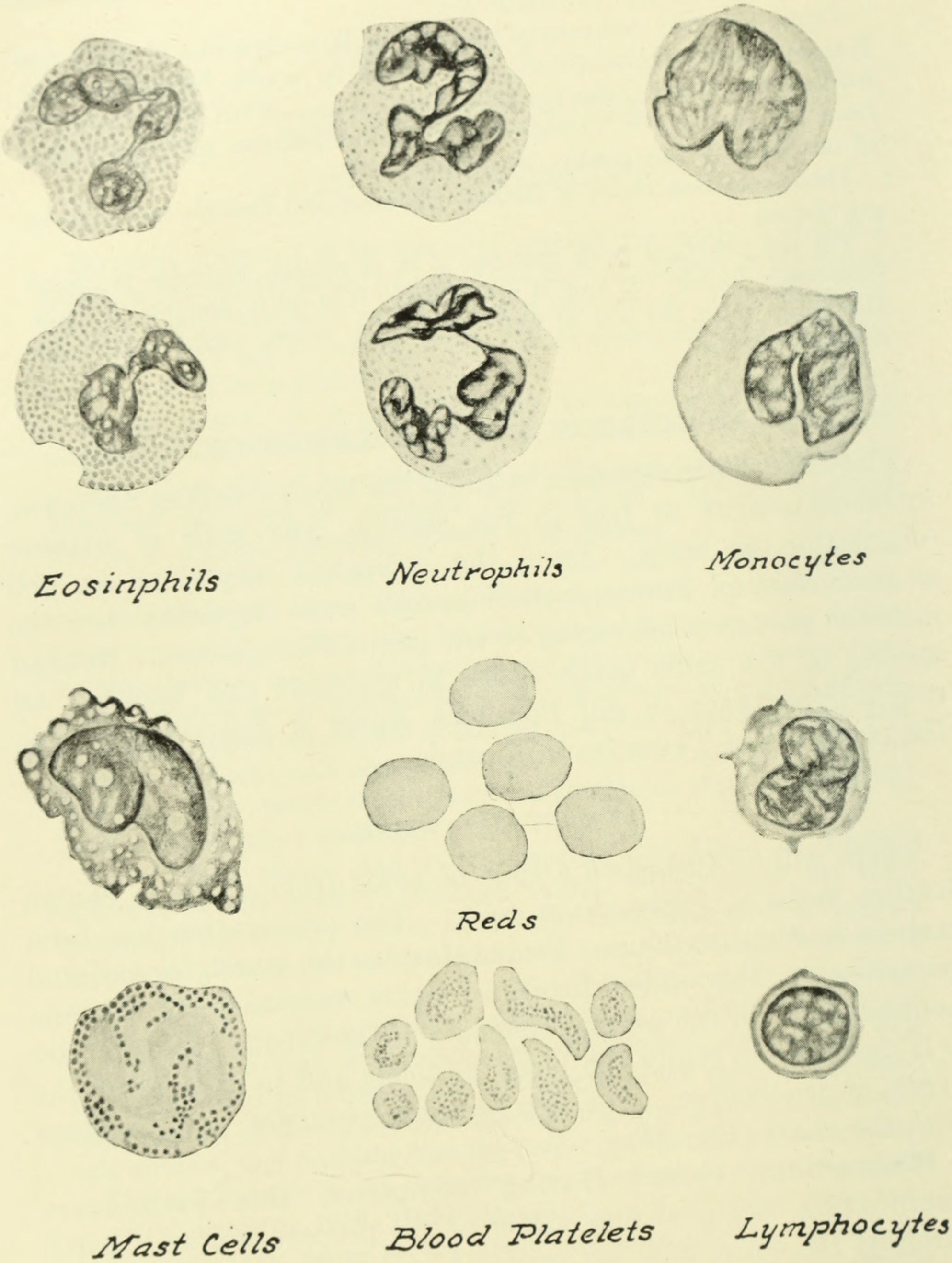
Eosinphils
Neutrophils
Monocytes
Reds
Mast Cells
Blood Platelets
Lymphocytes

Fig. 26.—Normal human blood. (*From chart by Dr. Hal Downey.*)

mosquitoes on successive days. In such cases parasites in various stages will be found on the one slide.

The parasite is injected into the blood of man by the bite of an Anopheles mosquito, in the form of an elongate veriform sporozoite. This stage will not be found in blood smears. The youngest stage to be noted is the signet ring stage, so called because the protoplasm consists of a ring staining blue, with a clear unstained space, and a red-staining spot, the nucleus, suggesting the seal of a ring. This growing stage is called, as in other Sporozoa, the *trophozoite.*

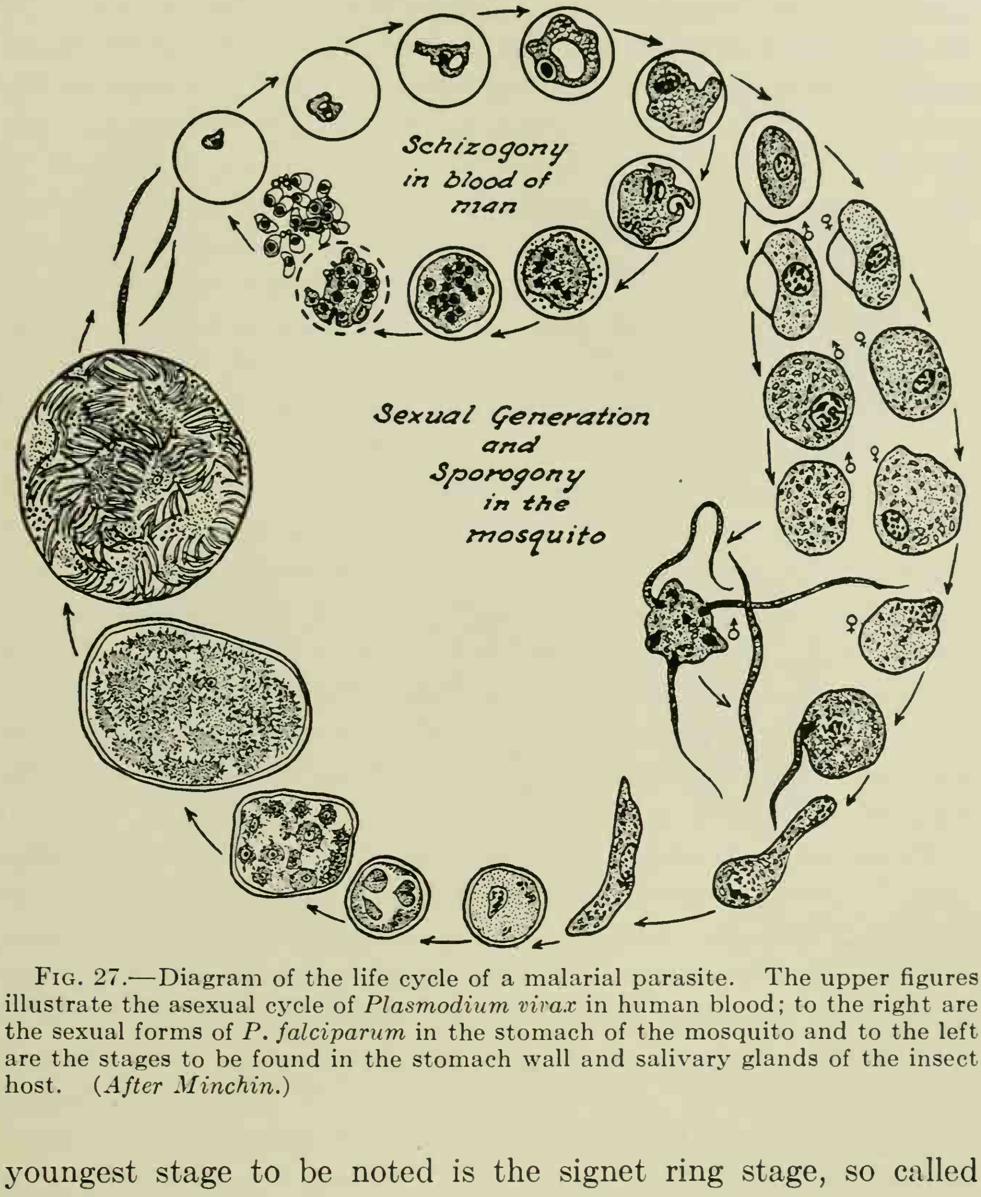

FIG. 27.—Diagram of the life cycle of a malarial parasite. The upper figures illustrate the asexual cycle of *Plasmodium virax* in human blood; to the right are the sexual forms of *P. falciparum* in the stomach of the mosquito and to the left are the stages to be found in the stomach wall and salivary glands of the insect host. (*After Minchin.*)

As the parasite grows, the ring of protoplasm enlarges and a dark brown pigment, derived from the hæmoglobin of the blood corpuscle, is deposited in it. This pigment makes it possible to detect the presence of malarial parasites even in freshly drawn and unstained preparations. It is characteristic of infections with *Plasmodium vivax* that the infected corpuscles become enlarged as the parasite develops. Not infrequently they become stippled with deep-staining granules, the Schüffner's dots. In the older parasites the dark pigment becomes more concentrated and the red-staining chromatin divides into small masses near the periphery. These with a small amount of protoplasm become the asexual spores or *merozoites*. In *P. vivax* they number 15 to 25, or thereabouts. The corpuscle ruptures and the merozoites, along with the pigment and a little residual protoplasm, escape into the blood stream. Such of the merozoites as are not destroyed by phagocytes re-enter red corpuscles and thus continue the asexual phase, or *schizogony*. The entire cycle from sporulation to the next escape of merozoites is undergone in 48 hours.

The sexual cycle, or *sporogony*, is completed in mosquitoes of the genus Anopheles, but the *gametocytes* are to be found in the human blood after the disease has progressed for some days. They can be distinguished from the schizonts by the fact that in the full-grown stage the chromatin forms one mass instead of being broken up preparatory to formation of merozoites. In the macrogametocyte this mass is compact and excentrically placed and the pigment consists of long rods. In the microgametocyte the chromatin is centrally placed and more diffuse, and the pigment is in small rods.

Plasmodium falciparum.—Preparations from a case of malignant malaria, caused by *Plasmodium falciparum*, should be compared with the above. As a rule only the ring stages and the mature gametocytes are found in the peripheral blood, the schizogony occurring in the internal organs of the host. There will be demonstrated sections of the brain from a fatal case of malignant malaria showing the schizonts in the capillaries. The gametocytes are very characteristic crescent or sausage-shaped forms. The macrogametocyte has its chromatin in a single mass in the center and the pigment clumped, while the microgametocyte stains less deeply, has more diffuse chromatin, and has its pigment scattered.

Plasmodium malariæ.—A third species of malarial parasite, *Plasmodium malariæ*, is the cause of quartan malaria of man. The schizogony is completed in 72 hours. The infected corpuscles are not enlarged and the number of merozoites produced from a single schizont varies from 6 to 12. Specimens will be demonstrated if available.

Bird Malaria.—Several species of our native birds harbor malarial parasites (Fig. 28) which belong to the same genus, Plasmodium, as do those of man. The better known of these complete their asexual cycle in 24 hours. The gametocytes differ from the schizonts much as in the species affecting man.

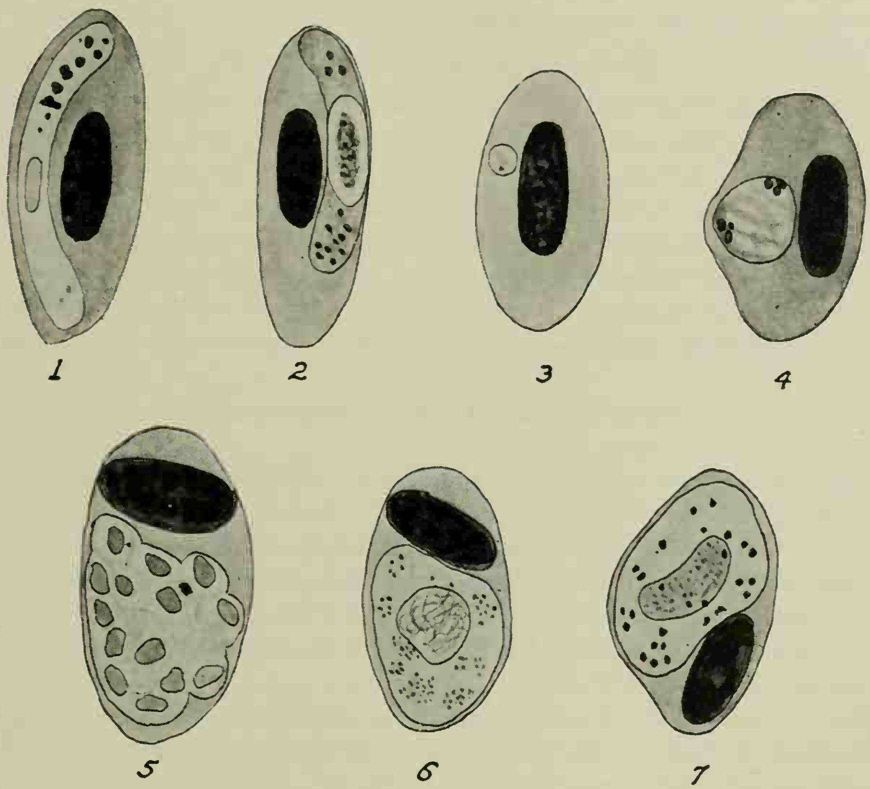

Fig. 28.—Malarial parasites from English sparrow. 1, microgametocyte of *Plasmodium præcox;* 2, macrogametocyte of same; 3–5, stages in the asexual development; 6, microgametocyte of *Plasmodium inconstans;* 7, macrogametocyte of same. (*After Hartman.*)

Study the demonstration specimens, making drawings of typical stages.

The sexual cycle of the malarial parasites is not readily studied in an introductory course. Such stages as are available will be demonstrated. They are most readily obtained in the case of the bird malarias which undergo their development in a number of species of mosquitoes, including *Culex pipiens*, the common rain-barrel species.

Piroplasmidæ.—These are minute hæmosporidians which, like the malarial parasites, inhabit the red blood corpuscles of various mammals but do not form the pigment which is characteristic of the malarial organism. They undergo an essential part of their life cycle in ticks and are transmitted by these arthropods to the mammalian host. There will be demonstrated (*Babesia bovis*) *Piroplasma bigeminum*, the cause of a widely distributed and highly fatal disease of cattle known in this country as "Texas fever." The organisms are minute pear-shaped bodies, usually two in a corpuscle. They vary in length from 2 to 4μ and in greatest width from 1.5 to 2μ. This species was the first of the group to be studied in detail and the first in which was demonstrated the transmission of a protozoal disease by an arthropod.

References

Good discussions of the malarial organisms of man are to be found in any modern textbook of parasitology and in many of the medical texts. The student who has access to a good library is advised to become acquainted with the epochal early researches on the transmission of malaria by mosquitoes.

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CHAPTER XVII

THE SARCOSPORIDIA

TECHNICAL SUGGESTIONS

For the study of Sarcosporidia, small fragments of the muscle of pigs should be examined under pressure as in the search for trichinæ. The small cysts of *Sarcocystis miescheriana* are not infrequently mistaken by beginners for those of the worm. They may be crushed and examined in the fresh condition for study of the spores. For sections, heavily infected muscle is pinned out in an extended condition and fixed for a half hour or more in picro-formol solution or in Schaudinn's sublimate alcohol. If the latter is selected metal pins must, of course, be avoided. Sections should be cut at 5μ and at 10μ and stained in hæmatoxylin and eosin, or, for details, in iron hæmatoxylin.

In many localities *Sarcocystis tenella* is readily obtained from the œsophagus of sheep. Hartmann recommends feeding the finely cut cysts in as fresh condition as possible to laboratory mice some two or three months before needed. The cysts will be particularly numerous in the muscles of the abdomen and of the legs of the infected mice.

In connection with this practicum there may be demonstrated spores of the *Myxosporidia* and *Microsporidia*, forms which can hardly be studied in detail but which the student should be able to recognize. We have found a *Leptotheca* from the kidneys of *Rana pipiens* a convenient and readily available illustration of the Myxosporidia, while *Nosema apis* in the ventriculus of the honey bee is probably the most widely distributed of the Microsporidia in this country. The more famous *Nosema bombycis* can be obtained by purchase, if not otherwise available. Abundant material from other sources is available in every locality, and the important monographs of Kudo, 1919 and 1924, should be available in every laboratory.

CHARACTERISTICS OF THE SARCOSPORIDIA

The **Sarcosporidia** are parasitic in the muscles of vertebrates, particularly mammals, and in a few cases have been reported for man. They are also recorded for birds and reptiles. Many of them are visible to the naked eye as whitish streaks or granules, often called *Miescher's tubes*, in the muscle fibers. Within these cysts are great numbers of thin-walled, sickle-shaped bodies called *spores*, although they are not homologous with the spores of other Sporozoa. Indeed the group is an aberrent one which, for reasons that will be apparent when sections are studied, has even been regarded by some authorities as of metozoan rather than protozoan relationship. Practically nothing is known of the life history.

PRACTICAL WORK

Sarcocystis miescheriana is commonly found in the muscles of swine. Examine under pressure fragments of infected pork and note the small, white, elongated cysts, or "Miescher's tubes," measuring from 2 to 3 mm. Dissect out some of these cysts and, crushing the thick enveloping membrane, note the numerous sickle-shaped spores.

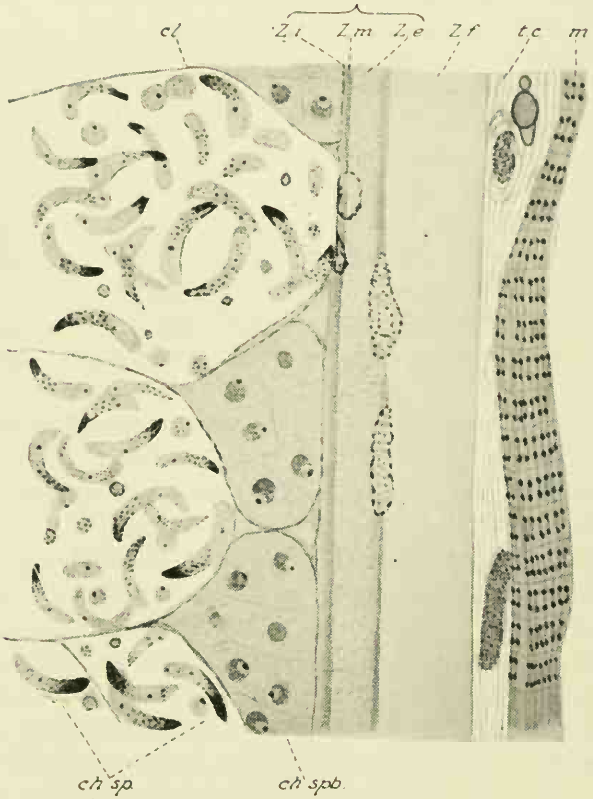

FIG. 29.—Longitudinal section of a cyst of *Sacrocystis tenella* from the œsophagus of a sheep. Cyst wall; *Z.e.*, *Z.m.*, *Z.i.*, external, mid, and inner layers; *Z.f.*, fibrous zone; *t.c.*, connective tissue; *m.*, muscle; *ch.sp.* chambers filled with spores; *ch.spb.*, chambers with sporoblasts. (*After Alexeieff.*)

Examine sections of muscle containing cysts of this species, or of *Sarcocystis tenella* of the sheep. Selecting a well-developed cyst, note that it is divided by membranous walls into a great number of chambers. The more central of these are empty, but the peripheral ones are filled with masses of the deep-staining, thin-walled falciform spores. The most peripheral chambers are filled with rounded cells, the *pansporoblasts*, from which the spores are developed. The enveloping membrane is regarded by some workers as derived wholly from the sarcoplasm of the infected muscle fiber. In favorable preparations there may be distinguished three layers: a delicate internal layer which passes inward to form the walls of the chambers, a compact middle layer, and a broad external layer (see Fig. 29).

Search your preparation for young stages of the parasite in the still distinctly recognizable muscle fiber.

Cnidosporidia.—In the superorder Cnidosporidia are placed Sporozoa infecting chiefly invertebrates and cold-blooded vertebrates, which are peculiar in that spore formation takes place in the trophozoite while it is still growing. Still more characteristic is the fact that the spores possess one or more peculiar bodies known as *polar capsules.* Within these capsules there is an elongate polar filament which is extruded when the spore is taken into a new host and presumably serves to anchor it while the amœboid organism of the spore makes its way into the tissues. Note the available demonstration specimens.

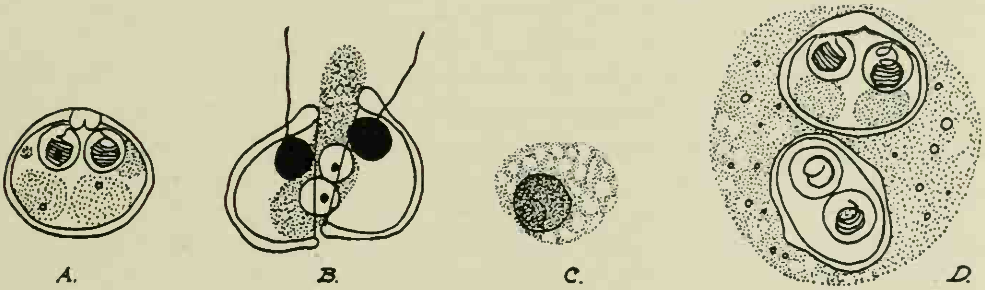

FIG. 30.—A myxosporidian, *Leptotheca ohlmacheri*, from the kidney of the frog. *A*, spore; *B*, emerging amœbula; *C*, amœbula whose nuclei are undergoing fusion; *D*, trophozoite containing two spores. (*After Kudo.*)

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CHAPTER XVIII

EXAMPLES OF PARASITIC INFUSORIA

TECHNICAL SUGGESTIONS

Our native frogs furnish the most convenient single source of material for the study of parasitic Protozoa. Representatives of the genera *Nyctotherus* and *Opalina* are almost always present and a species of *Balantidium* is not uncommon. The examination of living material should be supplemented by study of permanent mounts.

Motile and encysted specimens of *Balantidium* are readily obtainable by examining fresh fecal material from hogs or by bringing to the laboratory freshly removed sections of the cæcum and colon of hogs from slaughter houses. The organisms will remain alive for days at room temperature. Sections of heavily infected specimens will show numerous parasites *in situ*. Guinea-pigs occasionally harbor representatives of this genus, as do also cockroaches.

Where there is opportunity, some of the bizarre forms of ciliates from cattle and other ruminants should be demonstrated.

CHARACTERISTICS OF THE INFUSORIA

The **Infusoria** are Protozoa of definite form whose locomotory apparatus is constituted by a more or less considerable number of vibratile cilia on the surface of the body and which typically possess two forms of nuclei—a vegetative *macronucleus* and a much smaller reproductive *micronucleus*. In the subclass Opalinata the two or more nuclei present are of a single type. The transfer to new hosts is through formation of cysts. These may be purely protective, but in some forms multiplication occurs.

Many parasitic Infusoria are to be found in the higher animals, but in so far as is known they do not usually play an important role as pathogens. One species, *Balantidium coli*, is a widespread and serious parasite of man. Several species occur as ectoparasites of fish and are often the cause of serious epizoötics. As an introduction to the group, we shall study the infusorian parasites of the frog which harbors representatives of two genera, *Nyctotherus* and *Balantidium*, reported as infecting man.

PRACTICAL WORK

Open a freshly killed frog and mount in a drop of physiological salt solution (0.6 per cent NaCl) a bit of the black fecal content of the rectum. The infusoria are so large that they can be seen with the naked eye as actively moving, minute specks. Of these the more opaque, oval forms will probably prove to be *Nyctotherus cordiformis*, which we shall take as a type.

Nyctotherus cordiformis (Fig. 2) is a heterotrichous form; that is, it possesses two types of cilia. Covering the entire cell are parallel rows of short, similar cilia; but in addition there is an "adoral zone" of large, coarse cilia, or membranelles, which form a peristome extending from the anterior end along the side to the mouth, or cytostome, and into the so-called *cytopharynx*. Note the movement of these cilia and of those covering the general body surface. Near the posterior end is a single contractile vacuole and at the end a *cytopyge* through which excreta are discharged. In stained preparations note the large bean-shaped macronucleus and close to it, on the hinder concave side, the small micronucleus. Make a drawing showing the above features.

Balantidium entozöon (Fig. 2) is less common than *Nyctotherus cordiformis* in our native frogs but may be found. It is smaller and may be distinguished by the smaller peristome which is at the anterior end instead of extending halfway down the side. The important *Balantidium coli* of man and of the pig will be studied as a type of the genus.

Opalina (Fig. 31) species are the most common of the ciliates of frogs and toads. They are large, much-flattened Protozoa with a uniform covering of cilia and without a cytostome. The most characteristic feature is the presence in the adult specimens of many nuclei of a uniform type. Reproduction in the adult state is by oblique division, but during the spring months there is adaptation to parasitic life in the form of the production of numerous minute individuals which encyst and pass out with the feces of the frog. These cysts are taken up by tadpoles and liberate microgametocytes and macrogametocytes in the rectum of their new host. These cells give rise to gametes which conjugate, forming zygotes which develop into the multi-nucleate opalinid.

Study motile and stained preparations of the stages available.

Balantidium coli, a cause of dysentery in man, is also commonly found in pigs. If living specimens are available, note the distribution and the action of the two types of cilia. The peristome is a slitlike depression at the anterior end leading into the mouth, or cytostome. In the living specimen two contractile vacuoles may be seen, in addition to numerous food vacuoles.

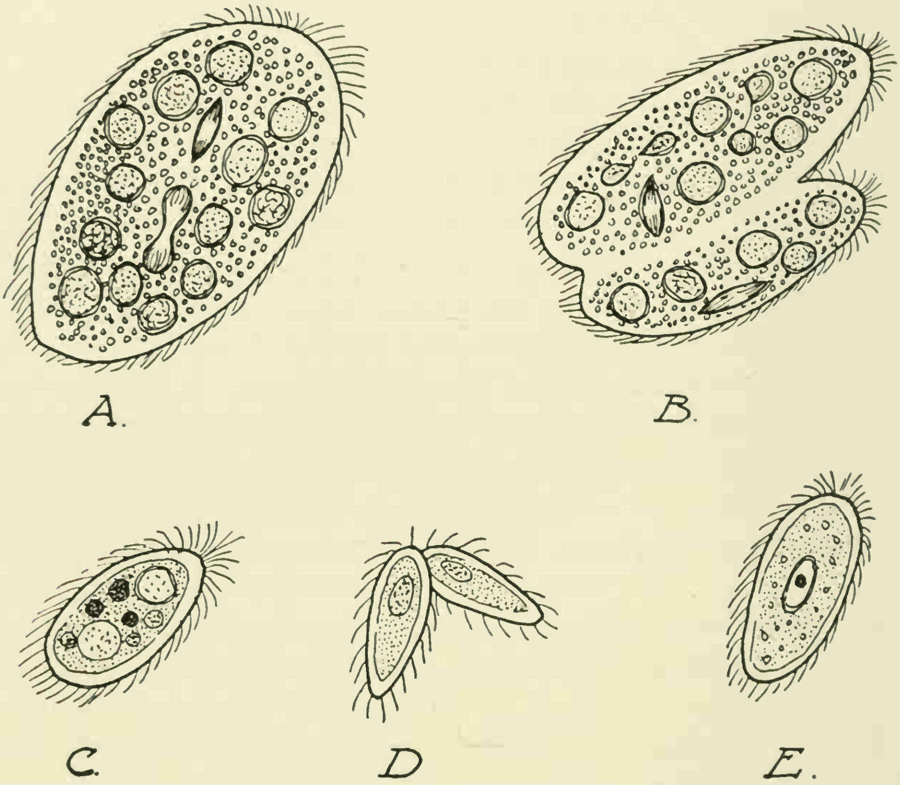

FIG. 31.—The life history of *Opalina ranarum*, after Neresheimer. *A*, multinucleate stage; *B*, oblique fission as it occurs in summer, fall and winter; *C*, gametocytes produced by repeated divisions in the springtime, encyst, are taken up by tadpoles and produce, *D*, gametes which copulate and form cysts from which escape; *E*, the uninucleate infusorian which develops into *A*.

At the posterior end is the anus, or cytopyge. There is a bean-shaped macronucleus and a rounded micronucleus.

Reproduction is by transverse fission and also by conjugation and cyst formation. Search your preparation for the large spherical cysts with a thick outer wall. Examine demonstration sections of the large intestine showing balantidia *in situ*.

References

McDONALD, J. D., 1922. On *Balantidium coli* (Malmsten) and *Balantidium suis* (sp. nov.) with an account of their neuromotor apparatus. *Univ. Calif. Pub. Zoölogy*, **20**: 243–300.

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CHAPTER XIX

THE LITERATURE OF ANIMAL PARASITOLOGY

TECHNICAL SUGGESTIONS

Local conditions and facilities must determine the most suitable place in the course for this practicum and the library materials to be used. It is suggested that the various texts, indices, and journals be shown and their use discussed in the laboratory period and that the problems be assigned for report at the following period.

In the assignment of problems a check should be made in order to insure that they can be answered from available facilities.

There should be insistance upon a standard form of citation, whether it be that given as an example or some other approved form.

PRACTICAL WORK

One of the chief objectives of a college course is that of affording a key to the stores of past and current information on the subject. Especially in a rapidly growing field, such as parasitology, a knowledge of methods of keeping in touch with present-day work is essential. It is the object of the present practicum to give an introduction to the literature of animal parasitology and to call attention to important bibliographic aids.

Examine the publications available in the following groups, and make the report indicated. Follow carefully the procedure suggested by your instructor.

A. Textbooks.—Examine the textbooks of animal parasitology on exhibit and list at least one each of American, German, and French publication, according to author, date, title, edition if more than first, number of pages, publisher, and place of publication. *Example:*

Chandler, A. C., 1926. "Animal Parasites and Human Disease," 3d ed., xiii + 573 pp. John Wiley & Sons, Inc., New York.

B. Periodicals.—In this group will be placed certain of the important publications dealing extensively with parasitology. List at least three of these, giving title, volume number, year, publisher, and address. Look over carefully one number of each of those you have selected and list in formal order an important article dealing with some phase of the work of this course. *Example:*

Caldwell, F. C., and E. L. Caldwell, 1926. Are *Ascaris lumbricoides* and *Ascaris suilla* identical? *Jour. Parasitology*, **13** (2): 141–145.

C. Federal and State Publications.—An assortment of Federal and state publications of value from the viewpoint of parasitology will be made available. Note the source of each of these and the general type of subject matter.

D. Indices.—The most important of these keys to the literature of parasitology are the following:

Agricultural Index. Vol. 1, 1916——. New York.
Bibliographia zoologica. Vol. 1, 1896——. Leipzig.
Index Catalogue of Medical and Veterinary Zoology. 1902——. Washington.
Index Catalogue of the Surgeon General's Library. 1880——. Washington.
Index medicus. Vol. 1, 1879——. Combined with the following in 1927:
Quarterly Cumulative Index to Current Medical Literature. Vol. 1, 1916——. Chicago.
Zoological Record. Vol. 1, 1864——. London.

Using such of the above indices as are available in the library, give a complete reference to each of the topics assigned and indicate where the reference was found. *Examples:*

1. A paper by (Hall), published in (1923), dealing with (internal parasites of dogs and cats).
2. An important paper, or book, published in (1925) which discusses (in a comprehensive manner, human protozoology).
3. A paper published within the past 18 months dealing with (the fish tapeworm, Diphyllobothrium latum, in the United States).

E. Abstract Journals.—The more important abstract journals for the student of animal parasitology are the following:

Biological Abstracts. Vol. 1, 1926——. Philadelphia.
Centralblatt für Bakteriologie, Parasitenkunde, und Infektionskrankheiten. Vol. 1, 1887——. Jena. (Beginning with Vol. 31, 1902, the abstracts are published as a separate part.)
Experiment Station Record. Vol. 1, 1889——. Washington.
Institut Pasteur, Bulletin. Vol. 1, 1903——. Paris.
Review of Applied Entomology. Series *B.*, Medical and Veterinary. Vol. 1, 1913——. London.
Tropical Disease Bulletin. Vol. 1, 1912——. London.
Tropical Veterinary Bulletin. Vol. 1, 1912——. London.

Using two of the above aids, give a complete citation to an important paper for each of the years (1920 and 1926), dealing with a parasite of man or animals. Cite not only the original place of publication but also the page reference to the abstract journal.

In not less than 100 or more than 200 words, give the essential points of the articles chosen.

APPENDIX

COLLECTION AND PRESERVATION OF ANIMAL PARASITES

INTESTINAL PROTOZOA

The various intestinal Protozoa cannot be prepared satisfactorily by methods such as those used in blood or bacteriological work, which permit of drying at any stage. They must be fixed while still moist and carried through much the same procedure as that used for animal tissues in general.

A small amount of fresh fecal material is taken on an applicator stick or a glass rod, or a bit of intestinal mucosa or other tissue taken in forceps, and quickly smeared over a thoroughly cleaned slide. If a stool sample should be very fluid, it is desirable first to cover the slide with a thin film of egg albumen. The smear preparation should be thicker than for ordinary bacteriological work and should be passed immediately into the fixing fluid.

For general work a very convenient technique is that of fixing in Bouin's picro-formol (p. 113) for 10 to 30 minutes, washing in several changes of 70 per cent alcohol to remove the excess picric acid, rinsing in water, and staining for a few minutes in a weak solution of Delafield's hæmatoxylin. The intensity of the stain should be controlled by rinsing and examining in tap water. Then dehydrate in ascending grades of alcohol; pass from 80 per cent into a 0.1 per cent eosin in 95 per cent alcohol if a counterstain is wanted. Rinse in 95 per cent alcohol, clear in carbol-xylol, and mount in balsam.

A standard and, once mastered, the best method for preparation of permanent mounts is that of fixation in warm Schaudinn's fluid and staining in iron hæmatoxylin.

Schaudinn's fluid is composed of 2 parts of saturated aqueous solution of corrosive sublimate, and 1 part of 95 per cent ethyl alcohol, acidulated by the addition of glacial acetic acid. A convenient quantity is:

Saturated aqueous solution of corrosive sublimate....	65 cc.
95 per cent ethyl alcohol..........................	33 cc.
Glacial acetic acid................................	2 cc.

It should be heated until steam is given off (60 to 70°C.) immediately before using. Handling slides with forceps or allowing metal to come in contact with the solution in any way must be avoided or precipitates will ruin the preparations. The various stages may be outlined as follows:

1. Prepare smear as above described.
2. Fix in warm Schaudinn's fluid, 15 minutes.
3. Rinse in 50 per cent alcohol, 3 to 5 minutes.
4. Transfer to 70 per cent alcohol to which has been added enough tincture of iodine to give a bright straw color, 10 minutes. If the fluid is bleached, renew it.
5. Harden in 95 per cent alcohol, 1 hour or more.
6. Seventy per cent alcohol, 5 minutes.
7. Fifty per cent alcohol, 5 minutes.
8. Rinse in distilled water.
9. Mordant in a 4 per cent solution of clear violet crystals of iron alum (ammonic-ferric sulphate) in distilled water, 6 hours.
10. Rinse in water.
11. Stain in 0.5 per cent ripened solution of hæmatoxylin in distilled water, 6 hours to overnight.
12. Rinse in water.
13. Differentiate in 2 per cent iron alum solution, controlling by rinsing in water and examining under the microscope from time to time. The background should be grayish and it is desirable to differentiate the slides to a slightly different degree since it is not feasible to search long for cysts as controls.
14. Wash in running water, or several changes, 20 minutes.
15. Dehydrate, in 50, 70, 95 per cent and absolute alcohol, 5 minutes each.
16. Clear in xylol, 5 minutes.
17. Mount in Canada balsam.

Instead of Canada balsam, euparol may be used as a mounting medium and the material passed directly from 95 per cent alcohol. Considerable economy results from the elimination of absolute alcohol and xylol.

Although the above method of preparing intestinal Protozoa is tedious and time consuming, it usually gives better results for inexperienced workers.

Kofoid and Swezy, 1925, recommend a rapid method through the use of alcoholic solutions: The stock solution 0.5 per cent iron hæmatoxylin is diluted with 10 parts of 70 per cent alcohol, and the stock solution of 4 per cent iron alum is diluted with 10 parts of 50 per cent alcohol. The iron alum will not remain long in solution in alcohol; hence the solution must be renewed frequently. After the usual fixation in Schaudinn's fluid, followed by ascending grades of alcohol, preparations are mordanted in the iron alum for 10 minutes, rinsed in 50 per cent alcohol, and stained for 10 minutes or longer. One hour gives better results for chromatin staining. After staining, decolorize in iron alum and wash in 50 per cent alcohol, or in water, for two hours.

Reference

Kofoid, C. A., and Olive Swezy, 1925. Mitosis and multiple fission in trichomonad flagellates. *Proc. Am. Acad. Arts Sci.*, **81** (6): 289–378, Fig. 104.

CESTODA

Collection.—For obtaining the smaller cestodes Meggitt uses the following technique:

Open the intestine in lengths of approximately 4 inches, one portion at a time, cut off the part opened and shake vigorously in a flat dish (a convenient size is 10 by 6 by 2.5 inches) filled with tepid water of approximately 40°C. temperature; if the intestinal content be not fresh and consequently contains much mucus, a slightly higher temperature should be employed. Salt solution or cold water should never be used . . . After washing in this manner, the intestine with the attached worms should be removed to another similar dish and examined with a powerful lens, preferably with a binocular, the scolices and smaller worms being dissected out with needles.

Rapid Examination.—For rapid examination the living scolex may be mounted directly from water into lacto-phenol. Mounted in this way the hooks are clearly visible and can be accurately studied. Meggitt advises ringing the cover slip with a mixture of Canada balsam and hard wax, melted, and applied with a glass rod.

For more permanent mounts harden the scolex in several changes of 95 per cent alcohol, clear in carbol-xylol, and mount in balsam.

The hooks of most tapeworms can be isolated in water by teasing the scolex apart under a binocular microscope. With the aid of a small, finely pointed camel's-hair brush touched to Buxton's fluid to make it adhesive the hooks can be transferred directly to a slide and mounted in Buxton's mounting medium (p. 112).

For the examination of the genital organs staining is necessary. Meggitt, 1924, recommends: 97 parts of a saturated solution of carmine in 45 per cent acetic acid, and 3 parts of a saturated solution of ferric acetate in glacial acetic acid, prepared immediately before use. The tapeworm is removed from a dish of water and placed alive in the stain for 5 to 30 minutes according to size. It can then be mounted directly in lacto-phenol or, preferably, placed for 5 minutes in absolute alcohol and then cleared in clove oil.

Killing and Fixation.—Many methods are recommended for killing tapeworms in an extended condition. Large tapeworms present the greatest difficulty since the entire chain must be killed instantly to prevent contraction. A method commonly employed (Baylis, 1922) is to pick up the worm by its caudal end allowing it to stretch to its full length before immersing it quickly into the fixative (Zenker's fluid, Bouin's fluid, or hot 70 or 80 per cent alcohol). It should then be dipped several times into the solution to insure uniform fixation.

Good results are likewise obtained by wrapping the specimen around one end of a glass plate and immersing it quickly into the fixative. Using this method spontaneous contractions which usually result are averted. Small specimens may be killed to advantage by compressing them between two glass slides and pouring the fixative over them.

Specimens killed in alcohols may be transferred directly into fresh 70 or 80 per cent alcohol for preservation. Those killed in Zenker's fluid should be left in the solution from 6 to 24 hours, and washed in running water for 12 to 24 hours. They should then be transferred to 35, 50, and 70 per cent alcohols, some 20 minutes in each. Add to the 70 per cent alcohol sufficient iodine to give a yellowish color, to remove mercuric crystals which might be present. Iodine is added as long as the solution continues to bleach. Specimens are then transferred to several changes of 70 or 80 per cent alcohol to wash out the iodine. If Bouin's fluid is used, fix for 6 to 14 hours, depending upon the size of the specimen, and then wash in 50 or 70 per cent alcohol until the color resulting from the picric acid is removed. Preserve in 70 or 80 per cent alcohol.

Staining and Mounting.—The most satisfactory stains are Delafield's hæmatoxylin, Ehrlich's acid hæmatoxylin, borax-carmine, and paracarmine. For staining in borax-carmine or in paracarmine specimens are transferred directly from 70 per cent alcohol. The hæmatoxylins are aqueous solutions which necessitate hydration to water before staining. The usual grades used are 70, 50, 35 per cent and water.

Stain intensity is largely a matter of the particular structures to be brought out. The best results are obtained by overstaining and differentiating in acidulated alcohol (2 per cent HCl in 70 per cent alcohol). Large specimens may be left in the stain overnight; for smaller specimens an hour will suffice.

Dehydration is accomplished by passing specimens through a series of graduated alcohols: 70, 80, 95 per cent. Large specimens should be left in each grade for an hour, with a change to fresh 95 per cent alcohol for a second hour. They are then transferred to carbol-xylol until thoroughly cleared, and mounted in Canada balsam or damar.

In mounting, each slide should include scolex, mature and gravid segments, and developing segments.

Sectioning.—Satisfactory methods of sectioning are included in all manuals on microscopic technique. For general studies sections may be made from specimens stained *in toto*. Detailed histological studies necessitate staining after sectioning. The usual paraffin method is most suitable.

References

BAYLIS, H. A., 1922. Notes on the collection and preservation of parasitic worms. *Parasitology*, **14**: 402–408.

MEGGITT, F. J., 1924. On the collection and examination of tapeworms. *Parasitology*, **16**: 266–268.

TREMATODA

Collection.—Trematodes are to be sought in a wide variety of locations in the animal body. Adults may occur in the liver, intestine, bladder, lungs, and blood vessels; while the encysted larval forms occur on the various organs, in the body cavity, intramuscularly, and, among other places, in the eyes of various hosts. Monogenetic species are to be found most commonly on the gills or at the base of the fins of various fish.

The presence of liver flukes is often most readily revealed by examination of the gall for eggs. Similarly scrapings of the trachea will betray the presence of lung trematodes (or nematodes). The liver should be cut into small pieces which are squeezed in water. Frequently the worms will emerge if the pieces are left standing in warm water. Trematodes of the digestive tract are often overlooked on account of their small size. The mucosa should be scraped into water and specimens sought with the aid of a microscope. The method of isolating adult trichinæ may be used to advantage, particularly if the water is near 38°C.

Killing and Fixation.—Trematodes may best be fixed in concentrated aqueous solution of corrosive sublimate plus 2 per cent of glacial acetic acid, or in picro-formol. Under field conditions it is often necessary to drop the specimens into 5 per cent formalin or into 70 per cent alcohol. If the latter is used, specimens may subsequently be softened by soaking in water.

For small species Looss' shaking method is the most generally useful. The specimens are put in a small vial nearly filled with physiological salt solution and are shaken violently for 3 minutes. One-half of the salt solution is then quickly poured off and replaced by an equal amount of the acetic-sublimate solution and the shaking continued for a minute or so. Then replace with acetic-sublimate solution and fix for 15 to 30 minutes, or up to several hours if desired. Wash in 50 per cent alcohol, for 15 to 30 minutes, then in 70 per cent plus sufficient iodine to give it a bright straw color. If the fluid is bleached, change or add more iodine. Too much iodine will injure the staining quality. It is desirable to leave in the iodized alcohol for an hour or more, before washing in clear 70 per cent and storing or staining.

Often specimens are advantageously killed under light pressure under the cover glass or between two slides held together by rubber bands or thread, while running the fixing agent under. The slide or cover should be lifted occasionally to insure access of the fluid to all parts. Too much pressure will dislocate organs. Mühling kills muscular forms in an extended condition by placing the specimen on a cover glass, cleaning off attached mucus, then quickly pressing over it a hot slide which has been wet with the fixing fluid. Fresh fluid is gradually added from the side, the cover being lifted from time to time.

Staining and Mounting.—Specimens may be stained overnight in very dilute Delafield's hæmatoxylin or in borax-carmine. If in the former they should first be washed in water, but if borax-carmine is used they should be transferred from 70 per cent alcohol. After staining wash in water and differentiate carefully in 70 per cent alcohol plus 2 per cent HCl, dehydrate in 80 per cent and 95 per cent for 15 to 30 minutes each, clear in carbol-xylol, and mount in balsam.

The periods given for the various operations are subject to wide modification, dependent on the size of the specimen and the convenience of the worker. Minute specimens can be carried through in 10 minutes each, but when using Delafield's hæmatoxylin as a stain it is always advisable to use a weak solution and stain for a considerable period. It should also be noted that if it is necessary to differentiate with acid alcohol after hæmatoxylin stain, it is desirable to follow by alcohol rendered slightly alkaline by the addition of a few drops of a 0.1 per cent solution of bicarbonate of soda, in order to prevent fading.

NEMATODA

Collection.—Adult nematodes are no less widely distributed than are trematodes. Though most commonly present in the alimentary tract, they should be sought in the liver, bladder, kidney, lungs, body cavity, and circulatory system. Larval forms are common in the tissues and organs.

Inconspicuous forms from the digestive tract are collected by slitting the various sections one at a time and washing their contents into tall jars of water to be repeatedly washed and sedimented until the fluid is clear. The sediment is then poured into shallow trays and carefully examined. In the meantime the wall of the section under study should be closely examined for adhering parasites. It should also be examined over a strong light for evidence of embedded forms.

Hall and Cram, 1926, point out the value of screening the contents of the digestive tract through a series of metal screens having mesh apertures 6, 12, 24, and 40 to the inch. These are used in a rack which is placed in a sink and washed with a stream of water, after which the material on the screens is examined for parasites. The lungs, urinary bladder, gall bladder, etc., should be slit open in a dish of water and also examined in this way. The method is of course applicable to forms other than nematodes.

Killing and Fixation.—The specimens found should not be subjected longer than necessary to the action of the water but should be vigorously shaken to clear them of adhering mucus and then killed in hot, almost boiling, 70 per cent alcohol to which, preferably, has been added 3 to 5 per cent of glycerine. The alcohol may be allowed to evaporate gradually or more rapidly in an oven at *ca.* 60°C. until they are left in cleared condition in pure glyercine. Care must be taken to insure that they are not exposed as the mixture evaporates.

The above-described method is not suitable for such forms as Trichosomum, Trichuris, and the like, since they become tightly coiled and shrink. Magath found that this could be prevented in great measure by placing the points of a pair of forceps together and working the specimens to and fro in the hot fluid as soon as placed in it. In applying this method the fluid should first be heated and then the worms transferred to it.

Nematodes which have been gradually brought into pure glycerine, as above, may be permanently mounted in glycerine jelly and the cover glass sealed with Noyer's cement or with very thick Canada balsam.

A simple and very satisfactory method of preservation and mounting recommended by Langeron is that of killing in 5 per cent formalin and transferring after several hours into lacto-phenol mixture. They may be mounted directly in this mixture and the cover glass sealed as above.

For preservation of histological detail Magath recommends killing in 50 per cent alcohol heated to 60 to 75°C. and transferring at once to a mixture of equal parts of alcohol, water, and acetic acid to which has been added 0.05 to 0.1 per cent osmic acid. In this the material is left for from 1 to 10 hours, depending on size.

The same author describes in detail a technique for infiltrating and mounting in Canada balsam, though this medium is thoroughly unsatisfactory for nematodes when the ordinary technique is attempted.

It should be noted that nematodes preserved in formalin or in alcohol can be cleared for temporary study by transferring to a mixture of 4 parts of melted carbolic acid crystals and 1 part absolute alcohol.

References

HALL, M. C., and E. B. CRAM, 1925. Some laboratory methods for parasitological investigations. *Jour. Agr. Research*, **30** (8): 773–776.

MAGATH, T. B., 1916. Nematode technique. *Trans. Am. Micro. Soc.*, **35** (4): 245–256.

SOIL NEMATODES

Various methods for the isolation of not only soil inhabiting parasitic stages but also free-living nematodes from the soil have been devised. The most used employs the Baermann apparatus as modified by Cort and his associates in hookworm work.

This isolation apparatus consists of a glass funnel, the outlet of which is closed by a clamp on a piece of rubber tubing. A fine meshed sieve with one or two layers of muslin cloth in the bottom is set in the funnel and on this the soil sample is placed. The funnel is then filled with water at blood heat to a point above the lower surface of the soil. The majority of the nematodes under these conditions pass into the water and can be collected. For extensive work a rack of suitable height is used with a battery of funnels held in place by padded crossbars. For many purposes an ordinary ring stand may be used to support

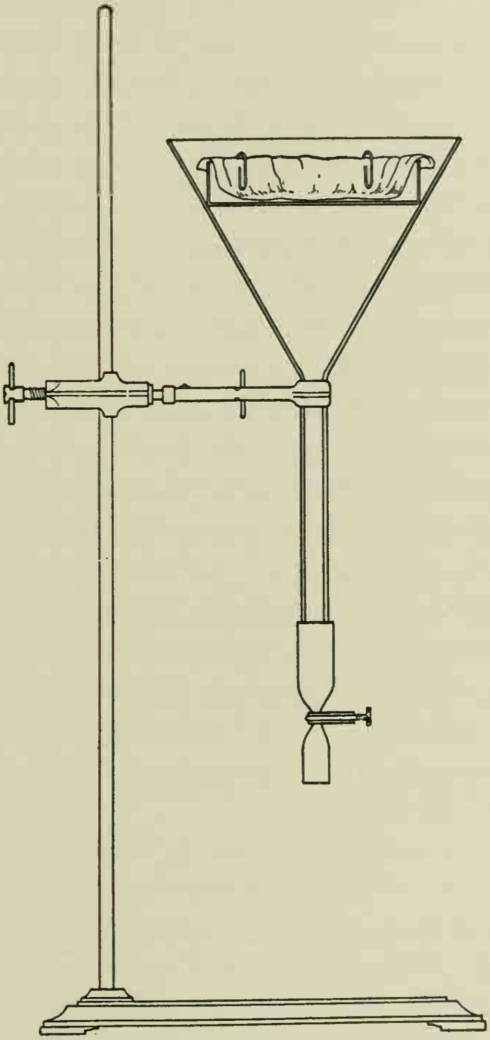

Fig. 32.—Baermann isolation apparatus for soil nematodes. (*Orig.*)

the apparatus, the funnel being held upright by ring clamps or burette clamps. In Fig. 32 is shown a simplified apparatus favorable for laboratory use, constructed of a 6-inch funnel, ½-inch rubber tubing closed by a screw clamp, and a 3½-inch sieve of 14-mesh brass screen.

A simplified apparatus as given by Sandground, 1925, consists only of a 5-inch funnel with a piece of fairly coarse cotton fabric attached to the rim by paper clips. This is allowed to sag about one inch into the funnel. The funnel is closed by a Hoffmann clamp on a piece of rubber tubing. Water with a temperature of from 104 to 108°F. is added until it covers the base of the fabric and then the culture is carefully added. Within little more than an hour the worms may be drawn from the funnel.

White, 1927, describes another very convenient method of isolating hookworm larvæ by trapping the migrating worms. Charcoal and feces are properly mixed in a large watch glass and transferred to the half of a Petri dish, with moistened filter paper in the bottom. Sterile water is poured into a crystallizing dish sufficient to cover the bottom and into it is placed the culture. A watch glass is used for a cover. Thus the larvæ reaching their third moulting stage, when they begin extensive migration, are trapped in the water surrounding the Petri dish. To collect them, the watch glass is removed and the Petri dish with the culture lifted out. The water containing the larvæ is poured into a test-tube, the supernatant fluid is removed, and a concentrate of the larvæ remains.

References

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Sandground, H. J., 1925. Some observations of the life-cycle, methods of diagnosis and incidence of *Strongyloides stercoralis* in the tropics. *Fourteenth Annual Report of the United Fruit Co., Med. Dept.*

White, G. F., 1927. A method for obtaining infective nematode larvæ from cultures. *Science,* **66** (1709): 302–303.

INTESTINAL TRICHINÆ

Intestinal, sexually mature trichinæ are usually recovered from experimental animals 2 to 10 days after a feeding of infected flesh, by slitting open the intestine and examining sections microscopically with the light well cut down. If their position in the mucosa is not being studied they may be recovered in considerable numbers by scraping the mucosa with the dull edge of a scalpel into a Petri dish with physiological salt solution.

To recover the adult worms in large numbers the following method has proved very satisfactory:

Starve a rat for 2 days and then feed it a piece of heavily trichinosed tissue about the size of the end of the thumb. Keep the animal unfed for 2 more days to eliminate intestinal debris, kill, and remove the intestine to a container filled with warm water.

Take short unslit pieces of the intestine and, holding with forceps over a vial filled with water, strip toward the free end with another pair. Apply sufficient pressure to remove all of the intestinal contents without tearing the intestine itself. Emulsify the extruded material by vigorously shaking to disentangle the worms from what little debris is present. Screen this out by emptying the vial on a 40-mesh screen placed over a stender dish filled almost to the rim with water. The screen should sag well below the water surface. The worms soon work their way through into the water and drop to the bottom where they may be recovered.

For permanent mounts kill specimens in hot 70 per cent alcohol. Change to fresh 70 per cent alcohol for preservation. Dehydrate gradually through the alcohols, clear in xylol, and mount in thin damar or balsam.

PERMANENT MOUNTS OF HELMINTH EGGS

Fecal material containing eggs or cysts of parasitic forms may be preserved in 10 per cent formalin solution, with or without previous sedimentation. The fluid should be changed occasionally as the offensive odor is greatly reduced by this procedure.

Langeron adds 10 per cent of glycerine as the eggs are thus rendered more transparent. They can be mounted in this fluid and sealed with Noyer's cement, but we have found that such preparations are not likely to be satisfactory for more than two or three years.

The best method of permanent mounting of helminth eggs in feces is that of Looss. A well-sedimented and sieved specimen with just enough water to make a thin paste is gradually added, with constant stirring, to hot, almost boiling 70 per cent alcohol plus 5 per cent glycerine, and allowed to cool. When cold the supernatant fluid is carefully poured off and replaced by fresh. The preparation is then put in an oven at 50°C. for a day or two, by which time the alcohol will have been evaporated and the fecal material evenly distributed through the glycerine. At laboratory temperatures this will, of course, require longer exposure and protection from dust. For mounts, a small drop of the sediment is placed in a small quantity of warm glycerine jelly on a slide, stirred carefully to distribute it, and the whole sealed with Noyer's lanoline cement.

PREPARATION AND STAINING OF BLOOD SMEARS

Preparation.—Slides and covers must be especially cleaned before using for the preparation of blood smears, or of fecal smears for the study of intestinal Protozoa. Pass slides over a flame to remove excess moisture and grease.

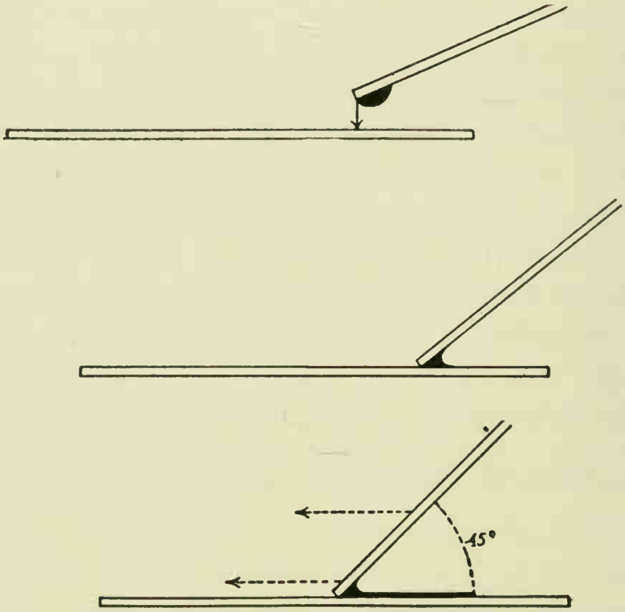

Fig. 33.—Method of making a blood film by pulling a drop of blood behind second slide. (*After Daniels.*)

Experience in the preparation of films and in the use of blood stains is best gained with human blood. Wipe the finger with alcohol and prick it with a sterile needle or spring lancet until free, easy drops issue forth. Wipe away the first drop with sterile cotton and select one a little larger than the head of a pin. Touch this with the end of a clean slide to be used as a "pusher" as illustrated by Fig. 33. Place this at an angle of 45 deg. near the end of a clean slide held horizontally. The drop should instantly spread along the width of the slide. Push the slide steadily forward at a moderate rate of speed so that the blood follows and spreads out in a thin film. The thickness of the film can be regulated by increasing or decreasing the angle at which the slide is held. Dry by waving it vigorously in the air. If a moist fixation is desired, the film should be fixed immediately, without drying, in Schaudinn's fluid, and treated as usual for that method (p. 99).

Staining.—To obtain the best results blood films should be stained promptly. Wright's blood stain is the most satisfactory for staining blood-inhabiting Protozoa as well as the blood cells. This is purchased in the form of a powder, 0.3 gram of which is dissolved in 100 cc. of acetone-free methyl-alcohol (absolute).

To confine the stain, mark across the slide at both ends of the film with a grease pencil or a bit of soft paraffin, place it on a level place, and with a pipette add about 20 to 30 drops of stain. Leave this for one minute, which tends to fix the blood, and then add distilled water, drop by drop, until a greenish metallic scum appears on the surface. The amount of water added is usually half the amount of the stain, or not more than an equal amount. Allow the stain to act for 2 to 3 minutes, rinse, and add distilled water for 2½ minutes or longer to differentiate the preparation. By watching the slide under a microscope the desired differentiation may be secured. Carefully blot dry.

A successful Wright's stain should show the following appearances of the blood cells: red cells, pink; nuclei of leucocytes, blue to violet; eosinophilic granules, red; mast granules, dark purple; neutrophilic granules, reddish lilac.

Blood smears may be mounted directly in balsam, after staining and drying, but are often dried and kept unmounted to be studied under immersion oil. Care should be taken to protect such slides from dust and from coming in contact with other objects. After use they should be flooded with xylol and very lightly wiped with lens paper or a bit of lint-free cloth to remove the cedar oil.

FORMULAS FOR REAGENTS AND MOUNTING MEDIA

Lacto-phenol.—Carbolic acid crystals c.p. 1 part; lactic acid, 1 part; glycerine, 2 parts; distilled water, 1 part. This discolors when exposed to the light, without affecting its value. It retains its colorless condition if kept in yellow bottles. Delicate forms may first be put in half-strength solution and transferred after a few hours to full strength.

Glycerine Jelly.—Glycerine, 100 parts; distilled water, 120 parts; gelatine, 20 parts; carbolic acid, melted crystals, 2 parts. Let the gelatine soak in water for a half hour and then dissolve with gentle heat. Add about 5 cc. of egg albumen and heat (not over 75°C.) for half an hour. Filter through moist hot flannel and add the glycerine and carbolic acid. Warm for 10 to 15 minutes stirring continually. For mounting place a drop of melted jelly in the center of a slide and transfer the object from an aqueous solution or glycerine to the jelly and arrange it. Lower a clean cover glass upon the object and gently press it down. After the jelly has set, clean away any excess around the cover and seal.

Buxton's Medium.—Distilled water, 50 cc.; glycerine, 20 cc., gum arabic, 40 grams; chloral hydrate, 50 grams; cocain hydrochlor., 0.5 grams. Dissolve gum in water, add chloral hydrate and cocain; when dissloved, add glycerine. Filter if necessary. This is a very useful medium in which objects can be mounted directly from water; alcohol specimens must be thoroughly washed in water. Buxton, who used it particularly in studies of mites, placed them in it on the slide in the living condition and found that they died rapidly in an extended condition.

Noyer's Lanoline Cement.—Langeron, who recommends this cement highly, gives the following directions: Anhydrous lanoline (mutton tallow), 20 grams, colophane (rosin), 80 grams. The commercial lanoline is slowly heated in an evaporating dish until the traces of water are driven off, and then the rosin is added, the mixture being stirred continually until the fusion is complete and perfectly homogeneous. Store in metal salve boxes. The cement is applied by means of a heated wire bent at a right angle some 22 mm. from the tip. Begin by putting a drop of the cement at each corner of the cover and then rapidly and smoothly applying to the edges. We have long used this cement with perfect satisfaction.

Apathy's Cement.—Equal parts of 60° paraffin and Canada balsam are heated together in a porcelain evaporating dish until the mass takes on a golden tint and no longer emits vapors of turpentine. Apply with a warm wire or glass rod.

Carbol-xylol.—This useful mixture will clear imperfectly dehydrated objects directly from 95 per cent alcohol and thus saves the expensive use of absolute alcohol. It consists of carbolic acid (melted crystals), 1 part; xylol, 3 parts.

Schaudinn's Fluid.—This invaluable fixing fluid for Protozoa consists of: saturated aqueous solution of corrosive sublimate, 65 parts; 95 per cent alcohol, 33 parts; glacial acetic acid, 2 parts. The corrosive sublimate solution is kept in stock and the mixture is made immediately before use. It may be used cold but better results are obtained by heating until steam begins to rise before putting the films into it. As with other corrosive sublimate mixtures, iodised alcohol should be used for washing.

Bouin's Picro-formol.—Saturated aqueous solution of picric acid, 30 parts; formalin, 10 parts; glacial acetic acid, 2 parts. Langeron, who regards this as a universal fixer, keeps a stock solution of 1 part of formalin and 3 of water saturated with picric-acid crystals. To this he adds 5 per cent of glacial acetic acid just before use. Tissues may be fixed for from 2 to 3 days to a week and are then placed in 80 per cent which is changed several times.

Brasil's Picro-formol is an alcoholic modification of Bouin's fluid which possesses great penetrating powers. Used warm, it is especially good for cysts. Eighty per cent alcohol, 150 cc.; formalin, 60 cc.; glacial acetic acid, 15 cc.; picric acid, 1 gram.

Zenker's Fluid.—Potassium dichromate, 2.5 grams; corrosive sublimate, 5 grams; water, 100 cc.; and add before using glacial acetic acid, 5 cc. Fix for 12 to 48 hours and wash in running water for an equal period, transfer to 70 per cent alcohol for 1 day, then to 80 per cent iodised alcohol to remove the corrosive sublimate. It is advisable to keep the tissues in the dark while in the alcohol.

Delafield's Hæmatoxylin.—Saturated aqueous solution of ammonia alum, 100 cc.; stock solution of hæmatoxylin (10 per cent in 95 per cent alcohol), 20 cc. Leave it exposed to the light and air in an unstoppered bottle for 3 or 4 days, filter, and add glycerine, 50 cc., and methyl alcohol, 50 cc. Let stand uncorked for a week or more, until the color is sufficiently dark; then filter. Solutions which have ripened for several months are best. For ordinary staining the solution should be diluted with 3 to 4 volumes of distilled water, but for staining tapeworms and flukes it may be so dilute as to have only a faint purple color and be allowed to act for 24 hours or more. If necessary to destain by the use of acid alcohol, follow this by alcohol made slightly alkaline by adding a few drops of 0.1 per cent sodium bicarbonate.

Grenacher's Borax-carmine.—Borax (4 per cent aqueous solution), 100 cc.; carmine, 3 grams. Boil until the carmine dissolves, dilute with an equal volume of 70 per cent alcohol, and filter after a day or so. Stain until penetration is complete, often requiring days, and differentiate with acidulated alcohol. For this 2 per cent of HCl in 70 per cent alcohol is often used, but better results are often obtained by using a weaker solution.

Heidenhain's Iron Hæmatoxylin (see p. 99).—Be sure that your mordant is made up from the clear violet crystals of the ferric alum (iron-ammonium-persulphate).

Wright's Stain.—This and the various other modifications of Romanowsky's stain are difficult to prepare and are best purchased from reliable dealers. Grübler's powdered form of Wright's stain has given us the best results. It should be dissolved at the rate of 0.3 gram in 100 cc. of absolute methyl alcohol (acetone free). For directions for use, see page 110.

THE MORE IMPORTANT ENDOZOA OF LABORATORY ANIMALS

The parasites discussed in the preceding outlines have been considered in their systematic relationships without special regard to their source. As an aid to the general examination of animals for endoparasites, the following lists of the more important species infecting laboratory animals and of the organs infected are given.

The papers cited are in most cases comprehensive reviews or contain important bibliographies.

CAT

PROTOZOA:
  Rhizopoda:
    Endamœba histolytica, intestine.
  Mastigophora:
    Giardia felis (*Giardia cati*), intestine.
    Trichomonas felis, intestine.
      felistomæ, mouth.
  Sporozoa:
    Eimeria felina, intestine.
    Isospora bigemina, intestine.
      felis, intestine.
      rivolti, intestine.
    Hepatozoön felis, leucocytes.

PLATYHELMINTHES:

Trematoda:

Alaria americana, intestine.
Ascocotyle minuta, intestine.
Clonorchis sinensis, liver.
Cotylophallus venustus, intestine.
Heterophyes æqualis, intestine.
  dispar, intestine.
  heterophyes, intestine.
Opisthorchis felineus, liver.
  pseudofelineus (*Amphimerus pseudofelineus*), liver.
  wardi, liver.
Parametorchis complexus, liver.

Cestoda:

Genus *Dipylidium*, the following species have been reported from the intestine:

trinchesei
pasquelei
chyzeri
œrleyi
caninum

Diphyllobothrium decipiens, intestine.
  latum, intestine.
  mansoni, intestine as adult, in musculature as plerocercoid.

Echinococcus granulosus, intestine as adult, cystic in liver, mesenteries, etc.

Mesocestoides lineatus, intestine.
Tœnia hydatigena, intestine.
  pisiformis (*Tœnia serrata*), intestine.
  tœniœformis (*T. crassicollis*), intestine.

NEMATHELMINTHES:

Nematoda:

Ancylostoma caninum, intestine.
  braziliense, intestine.
Capillaria aerophila, trachea, bronchi, lungs.
  lineare, trachea, bronchi, lungs.
Dirofilaria immitis, adults in heart, larvæ in circulating blood.
Ollulanus tricuspis, stomach, intestine.
Oxyuris compar, cœcum.
Toxascaris limbata, intestine.
Toxocara mystax (*Belascaris mystax*), intestine.
Trichinella spiralis, cysts in muscles, adults in intestine.

Acanthocephala:

Echinopardalis pardalis, probably intestine.

CHICKEN

PROTOZOA:

Rhizopoda:

Endamœba lagopodus, intestine.
Endolimax janisæ, intestine.

Mastigophora:

Chilomastix gallinarum, intestine.
Eutrichomastix gallinarum, intestine.

Sporozoa:

Eimeria avium, intestine.
Leucocytozoon smithi, blood.
Sarcocystis horvathi, muscles.

PLATYHELMINTHES:

Trematoda:

Cephalogonimus pellucidus, œsophagus.
Collyriclum faba, subcutaneous cysts on abdominal surfaces and in cloacal regions.
Echinostoma echinatum, intestine.
Mesogonimus commutatus, intestine.
Prosthogonimus pellucidus (?), bursa Fabricii.

Cestoda:

Amœbotænia sphenoides, intestine.
Davainea cesticillus (*Raillietina cesticillus*), intestine.
    echinobothridia (*R. echinobothridia*), intestine.
    tetragona, (*R. tetragona*), intestine.
    proglottina, intestine.
Hymenolepi carioca, (*Weinlandia*), intestine.

NEMATHELMINTHES:

Nematoda:

Ascaridia compar, small intestine.
    lineata, small intestine.
Cheilospirura hamulosa, gizzard.
Dispharynx spiralis, œsophagus, proventriculus,
Gongylonema ingluvicola, in mucous lining of the crop.
Heterakis gallinæ, cæcum.
Oxyspirura mansoni, under nictitating membrane, occasionally in nasal cavities and sinuses.
Subulura brumpti, cæca.
Syngamus trachealis, trachea.
Tetrameres americana, proventriculus.
Trichostrongylus tenuis, cæca and small intestine.

DOG

PROTOZOA:

Rhizopoda:

Endamœba histolytica, intestinal mucosa.

Mastigophora:
- *Giardia canis*, intestine.
- *Trichomonas canistomæ*, mouth.

Sporozoa:
- *Eimeria canis*, intestine.
- *Isospora bigemina*, intestine.
  - *felis*, intestine.
  - *rivolti*, intestine.
- *Hepatozoan canis*, red blood corpuscles.

PLATYHELMINTHES:

Trematoda:
- *Alaria americana*, intestine.
- *Ascocotyle italica*, intestine.
  - *minuta*, intestine.
  - *michiganensis*, intestine.
- *Centrocestus cuspidatus*, intestine.
- *Cotylophallus venustus*, intestine.
- *Cryptocotyle lingua*, accidental, normal parasite of birds.
- *Clonorchis sinensis*, liver.
- *Heterophyes æqualis*, intestine.
  - *dispar*, intestine.
  - *heterophyes*, intestine.

Cestoda:
- *Dipylidium*, the following species have been reported from the intestine:
  - *caninum* (*Tænia cucumerina*)
  - *œrleyi*
  - *sexcoronatum*
  - *walkeri*
- *Diphyllobothrium americanum*, intestine.
  - *cordatum*, intestine.
  - *latum*, intestine.
- *Dithyridium elongatum*, peritoneum in larval stage.
  - *martis*
  - *taxi*
- *Echinococcus granulosus*, intestine; cystic in liver, testes, spleen, vagina, pelvis, omentum, peritoneum.
- *Mesocestoides lineatus* (*Tænia lineata*), intestine.
- *Multiceps gaigeri*, intestine.
  - *serialis* (*Tænia serialis*), intestine.
  - *packi*, intestine.
  - *multiceps*, intestine.
- *Tænia*, the following species have been reported from the intestine:
  - *balaniceps*
  - *brachysoma*
  - *brauni*
  - *hydatigena*
  - *krabbei*
  - *ovis*

- *pisiformis* (*Tænia serrata*)
- *solium*, cystic, in muscles, eye, peritoneum, brain.

NEMATHELMINTHES:

Nematoda:

- *Ancylostoma caninum*, intestine.
  - *braziliense*, intestine.
- *Capillaria ærophila*, lungs, trachea, bronchi.
- *Dioctophyme renale*, kidney, body cavity.
- *Dirofilaria immitis*, circulating blood as larvæ, in heart tissue as adult.
- *Oslerus osleri*, respiratory passages.
- *Physaloptera rara*, intestine.
- *Spirocera sanguinolenta*, œsophagus, stomach.
- *Strongyloides stercoralis*, intestine.
- *Toxascaris limbata*, intestine.
- *Toxocara canis* (*Belascaris marginata*) intestine.
  - *vulpis* (*Belascaris vulpis*), intestine.
- *Trichinella spiralis*, cystic stage in muscle tissues; intestine as adults.
- *Trichuris vulpis*, cæcum.
- *Uncinaria stenocephala*, intestine.

Acanthocephala:

- *Onicola canis*, intestine.

FROG

PROTOZOA:

Rhizopoda:

- *Endamæba ranarum*, intestine.

Mastigophora:

- *Giardia agilis*, intestine.
- *Chilomastix caulleryi*, in intestine of tadpoles
- *Euglenamorpha hegneri*, rectum, tadpoles.
- *Hexamitus batrachorum*, rectum.
- *Phacus sp.*, rectum.
- *Polymastix bufonis*, rectum.
- *Trichomitus parvus*, rectum.
- *Trichomonas batrachorum*, rectum.
- *Trypanosoma batrachorum*, blood.

Sporozoa:

- *Diplospora lieberkühni*, kidney.
- *Eimeria ranæ*, intestine.
- *Lankestrella ranarum*, blood.
- *Leptotheca ohlmacheri*, tubules of kidney.
- *Pleistophora danilewski*, in mesenteries and muscles.

Infusoria:

- *Balantidium entozoon*, rectum.
- *Nyctotherus cordiformis*, rectum.
- *Opalina species*, rectum.

PLATYHELMINTHES:

Trematoda:

Agamodistomum sp., muscles of leg.
Cephalogonimus americanus, intestine.
Clinostomum attenuatum, encysted in muscles of legs and body wall.
Cystagora tetracystis (*Agamodistomum marcianse*), encysted in body cavity and throat.
Diplodiscus temperatus, rectum.
Glypthelmins quieta, intestine.
Gorgodera circava, urinary bladder.
  minima, urinary bladder.
Gorgoderina attenuatum, urinary bladder.
Halipegus occidualis, mouth, Eustachian tube.
Holostomum nitidum, intestine.
Loxogenes arcanum, encysted on viscera.
Megalodiscus ranophilus, rectum.
Monostomum ornatum, body cavity.
Pneumonœces coloradensis, lungs.
  complexus, lungs.
  longiplexus, lungs.
  medioplexus, lungs
  parviplexus, lungs.
  similiplexus, lungs.
Polystomum integerrimum, urinary bladder.
Tetracotyle pipientis, cystic in mesenteries and peritoneum also, in throat.

Cestoda:

Cylindrotœnia americanum, intestine.
Nematotœnia dispar, intestine.
Proteocephalus (*Ophiotœnia*) *sp.*, intestine.
Plerocercoid sp., intestine.

NEMATHELMINTHES:

Nematoda:

Agamonema (larvæ), body cavity.
Aplectana americana, cæcum.
  longicaudata, cæcum.
Foleyella americana, cysts in mesenteries.
Filaria sp., larvæ in blood.
Isociella solitaria, connective tissues.
Oswaldocruzia pipiens, intestine.
  subauricularis, probably intestine.
Pharyngodon batrachiensis, intestine of tadpole.
Rhabdias ranæ, lungs.

PIG

PROTOZOA:

Rhizopoda:

"*Endamœba coli*," intestine.
Endamœba histolytica (?), intestine.

"*Endolimax nana*," intestine.
Endamæba polecki, intestine.

Mastigophora:

Bodo sp., intestine.
Chilomastix mesnili (?) intestine
Trichomonas suis, intestine.

Sporozoa:

Eimeria debliecki, intestine.

Infusoria:

Balantidium coli, intestine.

PLATYHELMINTHES:

Trematoda:

Distomum maculosum, muscle.
Fasciola hepatica, liver.
Loossia romanicum, intestine.
Paragonimus westermani, lungs.
kellicotti, lungs.

Cestoda:

Echinococcus granulosus, cystic stage in practically every organ and tissue.
Multiceps multiceps, cystic stage in central nervous system.
Tænia hydatigena (*Cysticercus tenuicollis*), cystic stage in liver, free or attached to viscera in abdominal cavity.
solium (*Cysticercus cellulose*), cystic stage in musculature, eye, brain, liver, pancreas, spleen, and subcutaneous tissue.

NEMATHELMINTHES:

Nematoda:

Ancylostoma duodenale, intestine.
Arduenna strongylina, stomach.
Ascaris lumbricoides, (*A. suis*) intestine.
Bunostomum trigonocephalum, intestine.
Choerostrongylus brevivaginatus, bronchi and trachea.
Crassisoma urosubulatum, intestine.
Dioctophyme renale, kidney.
Filaria bauchei, lungs, pulmonary arteries.
Gongylonema ransomi, mucosa of tongue and œsophagus.
Hyostrongylus rubidus, stomach.
Metastrongylus elongatus, bronchi and trachea.
salmi, bronchi.
Necator americanus (?) intestine.
suillus, intestine.
Œsophagostomum dentatum, large intestine as adults, nodules in walls of intestine as larvæ.
Physocephalus sexalatus, stomach.
Setaria bernardi, peritoneal cavity and encysted on the surface of the liver.

Stephanurus dentatus, abdominal viscera.
Strongyloides stercoralis, intestine.
suis, intestine.
Trichinella spiralis, intestine as adults, cystic in the muscles as larvæ.
Trichuris ovis, cæcum and colon.
suis, cæcum and colon.
Uncinaria stenocephala, intestine.

Acanthocephala:

Macracanthorhynchus hirudinaceus (*Echinorhynchus gigas*), small intestine.

RABBIT

PROTOZOA:

Rhizopoda:

Endamœba cuniculi, intestine.

Mastigophora:

Chilomastix cuniculi, cæcum.
Embadomonas cuniculi, cæcum.
Enteromonas intestinalis, intestine.
Giardia duodenalis, intestine.

Sporozoa:

Eimeria perforans, intestine.
stiedæ, liver.

PLATYHELMINTHES:

Trematoda:

Fasciola hepatica, liver.
Hasstilesia texensis, intestine.
tricolor, intestine.

Cestoda:

Cittotænia ctenoides, intestine.
Multiceps packi, cœnurus stage in heart, probably not limited to that tissue.
Multiceps serialis, subcutaneous, axillary, intra-abdominal cavity, pleural cavity, etc., in cœnurus stage.
Tænia pisiformis, early cystic stages in liver, later as bladderworms in body cavity or as cysts attached to the mesenteries.

NEMATHELMINTHES:

Nematoda:

Graphidium strigosus, stomach.
Obeliscoides cuniculi, stomach.
Passalurus ambiguus, cæcum.
Trichuris leporis, cæcum and colon.

RAT

PROTOZOA:

Rhizopoda:

Endamœba decumani (*Councilmania decumani*), intestine.
histolytica, intestine.
ratti, intestine.

Mastigophora:

Chilomastix bettencourti, intestine.
Giardia lamblia (*G. intestinalis*), intestine.
  muris, intestine.
Trichomonas muris, intestine.
Trypanosoma lewisi, blood.

Sporozoa:

Hepatozoon muris (*Leucocytozoon muris*), leucocytes.

PLATYHELMINTHES:

Trematoda:

Ascocotyle (*Parascocotyle*) *diminuta*, intestine.
Echinostoma spiculator, intestine.
Heterechinostomum magniovatum, intestine.

Cestoda:

Catenotænia pusilla, intestine.
Hymenolepis, the following species from the intestine:
  contracta
  crassa
  nana (*H. murina*, *H. fraterna*)
  diminuta
  diminutoides
  horrida
  inexpectata
  microstoma
  relicta
Multicapsiferina guineensis, intestine.
Multiceps sp., larval stage in connective tissue.
Raillietina sp., intestine.
Tænia brachydera, intestine.
  hydatigena, larval stage in mesenteries of abdominal cavity.
  tæniæformis (*T. crassicollis*), cystic stage in liver.
Tetrathyridium bailleti (*Dithyridium*), peritoneum.
  (?) *elongatum*, peritoneum.

NEMATHELMINTHES:

Nematoda:

Heligmosomum muris, intestine.
Hepaticola hepatica, liver
Heterakis spumosa, cæcum.
Protospirura muris (*Spiroptera obtusa*), stomach.
  columbiana, stomach, æsophagus, intestine.
Strongyloides papillosus, intestine.
Syphacia obvelata (*Oxyuris obvelata*), cæcum and large intestine.
Trichinella spiralis, intestine as adults, cystic in muscles.
Trichosomoides crassicauda, bladder.

Acanthocephala:

Moniliformis moniliformis (*Echinorhynchus moniliformis*), intestine.

SHEEP

PROTOZOA:

Rhizopoda:
- *Endamœba ovis,* intestine.

Mastigophora:
- *Trypanosoma melophagium,* blood.
- *Embadomonas ovis,* rectum.

Sporozoa:
- *Babesia motasi,* blood.
    - *ovis,* blood.
    - *sergenti,* blood.
- *Eimeria faurei,* intestine.
    - *intricata,* intestine.
- *Globidium gilruthi,* mucosa of stomach.
- *Pneumocystis cornii,* lung tissue.
- *Theileria hirci,* peripheral blood, liver, and spleen.

Infusoria:
- *Balantidium sp.,* intestine.

PLATYHELMINTHES:

Trematoda:
- *Dicrocœlium dendriticum,* liver.
- *Fasciola hepatica,* liver.
- *Fascioloides magna,* liver.

Cestoda:
- *Echinococcus granulosus,* cystic stage in all organs.
- *Monezia expansa,* intestine.
    - *planissima,* intestine.
- *Tœnia hydatigena,* cystic stage attached to abdominal viscera.
    - *ovis,* cystic stage in muscles and on surface of various organs of viscera.
- *Thysanosoma actinoides,* intestine.
- *Multiceps multiceps,* cystic stage in central nervous system.

NEMATHELMINTHES:

Nematoda:
- *Bunostomum trigonocephalum,* intestine.
- *Dictyocaulus filaria,* bronchi, bronchioles.
- *Hœmonchus contortus,* stomach.
- *Nematodirus spathiger,* intestine.
- *Proteracrum columbianum,* larvæ in nodules on small and large intestines, adults in large intestine.
- *Synthetocaulus rufescens,* bronchioles and lung tissue.
- *Trichuris* (*Trichocephalus*) *ovis,* cæcum, large intestine.

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Biological Supply Service, Soochow University, Soochow, China.
Denoyer-Geppert C., 5235 Ravenswood Ave., Chicago, Illinois.
Établissement du Docteur Auzoux, 56 rue de Vaugirard, Paris (6), France.
Galigher, Albert E., 1057 Solono Ave., Berkeley, California.
General Biological Supply House, 761 E. 59th Place, Chicago Illinois.
Pacific Biological Laboratories, Pacific Grove, California.
Powers and Powers, Station A, Lincoln, Nebraska.
Schlüter and Mass, Naturwissenschaftliche Lehrmittel-Anstalt Halle a. S., Germany.
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